formamide. rinsing well in deionized H_2O, blowing dry, and storing at room temperature.

C. PREPARATION OF LABELED RNA/HYBRIDIZATION TO ARRAY

1. Tagged primers

The primers used to amplify the target nucleic acid should have promoter sequences if one desires to produce RNA from the amplified nucleic acid. Suitable promoter sequences are shown below and include:

(1) the T3 promoter sequence:

5'-CGGAATTAACCCTCACTAAAGG

5'-AATTAACCCTCACTAAAGGGAG;

(2) the T7 promoter sequence:

5' TAATACGACTCACTATAGGGAG;

and (3) the SP6 promoter sequence:

5' ATTTAGGTGACACTATAGAA.

The desired promoter sequence is added to the 5' end of the PCR primer. It is convenient to add a different promoter to each primer of a PCR primer pair so that either strand may be transcribed from a single PCR product.

Synthesize PCR primers so as to leave the DMT group on. DMT-on purification is unnecessary for PCR but appears to be important for transcription. Add 25 μl 0.5M NaOH to collection vial prior to collection of oligonucleotide to keep the DMT group on. Deprotect using standard chemistry -- 55°C overnight is convenient.

HPLC purification is accomplished by drying down the oligonucleotides, resuspending in 1 mL 0.1 M TEAA (dilute 2.0 M stock in deionized water, filter through 0.2 micron filter) and filter through 0.2 micron filter. Load 0.5 mL on reverse phase HPLC (column can be a Hamilton PRP-1 semi-prep, #79426). The gradient is 0 -> 50% CH_3CN over 25 min (program 0.2 μmol.prep.0-50, 25 min). Pool the desired fractions, dry down, resuspend in 200 μl 80% HAc. 30 min RT. Add 200 μl EtOH; dry down. Resuspend in 200 μl H_2O, plus 20 μl NaAc pH5.5, 600 μl EtOH. Leave 10 min on ice; centrifuge 12,000 rpm for 10 min in microfuge. Pour off supernatant. Rinse pellet with 1 mL

EtOH, dry, resuspend in 200 μl H2O. Dry, resuspend in 200 μl TE. Measure A260, prepare a 10 pmol/μl solution in TE (10 mM Tris.Cl pH 8.0, 0.1 mM EDTA). Following HPLC purification of a 42 mer, a yield in the vicinity of 15 nmol from a 0.2 μmol scale synthesis is typical.

2. Genomic DNA Preparation

Add 500 μl (10 mM Tris.Cl pH8.0, 10 mM EDTA, 100 mM NaCl, 2% (w/v) SDS, 40 mM DTT, filter sterilized) to the sample. Add 1.25 μl 20 mg/ml proteinase K (Boehringer) Incubate at 55°C for 2 hours, vortexing once or twice. Perform 2x 0.5 mL 1:1 phenol:$CHCl_3$ extractions. After each extraction, centrifuge 12,000 rpm 5 min in a microfuge and recover 0.4 mL supernatant. Add 35 μl NaAc pH5.2 plus 1 mL EtOH. Place sample on ice 45 min; then centrifuge 12,000 rpm 30 min, rinse, air dry 30 min, and resuspend in 100 μl TE.

3. PCR

PCR is performed in a mixture containing, per reaction: 1 μl genomic DNA; 4 μl each primer (10 pmol/μl stocks); 4 μl 10 x PCR buffer (100 mM Tris.Cl pH8.5, 500 mM KCl, 15 mM $MgCl_2$); 4 μl 2 mM dNTPs (made from 100 mM dNTP stocks); 1 U Taq polymerase (Perkin Elmer, 5 U/μl); H_2O to 40 μl. About 40 cycles (94°C 30 sec, 55°C 30 sec, 72°C 30 sec) are performed, but cycling conditions may need to be varied. These conditions are for 0.2 mL thin wall tubes in Perkin Elmer 9600. For products in the 200 to 1000 bp size range, check 2 μl of the reaction on a 1.5% 0.5xTBE agarose gel using an appropriate size standard. For larger or smaller volumes (20 - 100 μl), one can use the same amount of genomic DNA but adjust the other ingredients accordingly.

4. *In vitro* transcription

Mix: 3 μl PCR product; 4 μl 5x buffer; 2 μl DTT; 2.4 μl 10 mM rNTPs (100 mM solutions from Pharmacia); 0.48 μl 10 mM fluorescein-UTP (Fluorescein-12-UTP, 10 mM solution, from Boehringer Mannheim); 0.5 μl RNA polymerase (Promega T3 or T7 RNA polymerase); and add H_2O to 20 μl. Incubate at 37°C for 3

h. Check 2 μl of the reaction on a 1.5% 0.5xTBE agarose gel using a size standard. 5x buffer is 200 mM Tris pH 7.5, 30 mM $MgCl_2$, 10 mM spermidine, 50 mM NaCl, and 100 mM DTT (supplied with enzyme). The PCR product needs no purification and can be added directly to the transcription mixture. A 20 μl reaction is suggested for an initial test experiment and hybridization; a 100 μl reaction is considered "preparative" scale (the reaction can be scaled up to obtain more target). The amount of PCR product to add is variable; typically a PCR reaction will yield several picomoles of DNA. If the PCR reaction does not produce that much target, then one should increase the amount of DNA added to the transcription reaction (as well as optimize the PCR). The ratio of fluorescein-UTP to UTP suggested above is 1:5, but ratios from 1:3 to 1:10 - all work well. One can also label with biotin-UTP and detect with streptavidin-FITC to obtain similar results as with fluorescein-UTP detection.

For nondenaturing agarose gel electrophoresis of RNA, note that the RNA band will normally migrate somewhat faster than the DNA template band, although sometimes the two bands will comigrate. The temperature of the gel can effect the migration of the RNA band. The RNA produced from *in vitro* transcription is quite stable and can be stored for months (at least) at -20°C without any evidence of degradation. It can be stored in unsterilized 6xSSPE 0.1% triton X-100 at -20°C for days (at least) and reused twice (at least) for hybridization, without taking any special precautions in preparation or during use. RNase contamination should of course be avoided. When extracting RNA from cells, it is preferable to work very rapidly and to use strongly denaturing conditions. Avoid using glassware previously contaminated with RNases. Use of new disposable plasticware (not necessarily sterilized) is preferred, as new plastic tubes, tips, etc., are essentially RNase free. Treatment with DEPC or autoclaving is typically not necessary.

5. Fragmentation

Heat transcription mixture at 94 degrees for forty min. The extent of fragmentation is controlled by varying Mg^{2+} concentration (30 mM is typical), temperature, and duration of heating.

6. Hybridization, Scanning, & Stripping

A blank scan of the slide in hybridization buffer only is helpful to check that the slide is ready for use. The buffer is removed from the flow cell and replaced with 1 mL of (hydrolysed) RNA in hybridization buffer and mixed well. Incubate for 15 - 30 min at 18°C. Remove the hybridization solution, which can be saved for subsequent experiments. Rinse the flow cell 4 - 5 times with fresh changes of 6 x SSPE / 0.1% Triton X-100, equilibrated to 18°C. The rinses can be performed rapidly, but it is important to empty the flow cell before each new rinse and to mix the liquid in the cell thoroughly. A series of scans at 30 min intervals using a hybridization temperature of 25°C yields a very clear signal, usually in at least 30 min to two hours, but it may be desirable to hybridize longer, *i.e.*, overnight. Using a laser power of 50 μW and 50 μm pixels, one should obtain maximum counts in the range of hundreds to low thousands/pixel for a new slide. When finished, the slide can be stripped using warm water.

These conditions are illustrative and assume a probe length of ~15 nucleotides. The stripping conditions suggested are fairly severe, but some signal may remain on the slide if the washing is not stringent. Nevertheless, the counts remaining after the wash should be very low in comparison to the signal in presence of target RNA. In some cases, much gentler stripping conditions are effective. The lower the hybridization temperature and the longer the duration of hybridization, the more difficult it is to strip the slide. Longer targets may be more difficult to strip than shorter targets.

7. Amplification of Signal

A variety of methods can be used to enhance detection of labelled targets bound to a probe on the array. In one

embodiment, the protein MutS (from E. coli) or equivalent proteins such as yeast MSH1, MSH2, and MSH3; mouse Rep-3, and Streptococcus Hex-A, is used in conjunction with target hybridization to detect probe-target complex that contain mismatched base pairs. The protein, labeled directly or indirectly, can be added to the chip during or after hybridization of target nucleic acid, and differentially binds to homo- and heteroduplex nucleic acid. A wide variety of dyes and other labels can be used for similar purposes. For instance, the dye YOYO-1 is known to bind preferentially to nucleic acids containing sequences comprising runs of 3 or more G residues.

8. Detection of Repeat Sequences

In some circumstances, *i.e.*, target nucleic acids with repeated sequences or with high G/C content, very long probes are sometimes required for optimal detection. In one embodiment for detecting specific sequences in a target nucleic acid with a DNA chip, repeat sequences are detected as follows. The chip comprises probes of length sufficient to extend into the repeat region varying distances from each end. The sample, prior to hybridization, is treated with a labelled oligonucleotide that is complementary to a repeat region but shorter than the full length of the repeat. The target nucleic is labelled with a second, distinct label. After hybridization, the chip is scanned for probes that have bound both the labelled target and the labelled oligonucleotide probe; the presence of such bound probes shows that at least two repeat sequences are present.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. All publications and patent documents cited in this application are incorporated by reference in their entirety for all

purposes to the same extent as if each individual publication or patent document were so individually denoted.

Mutation	Exon	Ex Size	Pop Freq	Location	Sequence Around Mutation Site	PRIMERS	Amp Sz
297-3 C>T	3	109	Manchester	Sub C>T -3 Exon 3	CTTTTTATTCTTTTG(C>T)AGAGAATGGGATAGA	787/788	297
R75Q	3	109	Manchester	Substitute G>A at 60	TAATGCCCTTCGGCC(G>A)ATGTTTTTTCTGGA	787/788	297
300 del A	3	109	Manchester	Delete A at 4	ATTCTTTTTGCAGAGAaTGGGATAGAGAGCTGGCT	787/788	297
E60X	3	109	Manchester	Substitute G>T at 14	GAATGGGATAGA(G>T)AGCTGGCTTCAAAGA	787/788	297
L88S	3	109	Manchester	Substitute T>C at99	CTATGGAATCTTTTT(T>C)ATATTTAGGGGTAAG	787/788	297
G85E	3	109	0.70%	Substitute G>A at90	TTATGTTCTATG(G>A)AATCTTTTTATATTTAG	787/788	297
R117H	4	216	0.80%	Substitute G>A at 77	AACAAGGAGGAAC(G>A)CTCTATCGGCGATTTAT	851/789	381
R117C	4	216	rare	Substitute C>T at 76	AACAAGGAGGAAC(C>T)GCTCTATCGGCGATTTAT	851/789	381
Y122X	4	216	0.30%	Substitute T>A at 93	TATCGGCGATTTA(T>A)CTAGGCATAGGCTTATG	851/789	381
I148T	4	216	Fr Can (10%)	Substitute T>C at 170	GGCCTTCATCACA(T>C)TGGAATGCAGATGAGA	851/789	381
621+1G>T	14	216	1.30%	Sub G>T after last base	GATTTATAAGAAG(G>T)TAATACTTCCTTGCAC	851/789	381
711+1G>T	15	90	0.90%	Sub G>T after last base	CAAATTTGATGAA(G>T)TATGTACCTATTGATT	887/888	289
L206W	6a	164	Fr Can (10%)	Substitute T>G at 38	TGGATCGCTCCTT(T>G)GCAAGTGGCACTCCTC	934/935	331
1138 ins G	7	247	Manchester	Insert G at 137	AATCATCCTCCGGAAAGATATTCACCACCATCT	789/790	404
1154 ins TC	7	247	Manchester	Insert TC at 153	TATTCACCACCATCTCTCATTCTGCATTGTT	789/790	404
1161 del C	7	247	Manchester	Delete C at 160	CCACCATCTCATTCTG>ATTGTTCTGGGCATGG	789/790	404
R334W	7	247	0.40%	Substitute C>T at 131	AAGGAATCATCCTC(C>T)GGAAAATATTCATTA	789/790	404
R347H	7	247	0.10%	Substitute G>A at 171	CTGCATTGTTCTGC(G>A)CATGGCGGTCACTCG	789/790	404
R347L	7	247	rare	Substitute G>T at 171	CTGCATTGTTCTGC(G>T)CATGGCGGTCACTCG	789/790	404
R347P	7	247	0.50%	Substitute G>C at 171	CTGCATTGTTCTGC(G>C)CATGGCGGTCACTCG	789/790	404
1078 delT	7	247	1.10%	Delete T at 77	CTTCTTCTCAGGGTTCTTTGTGGTGTTTTTATC	789/790	404
1248+1 G>A	7	247	Manchester	Sub G>A 1 after Exon 7	AAACAAAATACAG(G>A)TAATGTACCATAATG	789/790	404
A455E	9	183	0.40%	Substitute C>A at 155	AGGACAGTTGTTGG(C>A)GGTTGCTGGATCCA	891/892	388
G480C	10	192	rare	Substitute G > T at 46	GGAGCCTTCAGAG(G>T)GTAAAATTAAGCACA	760/850	304
Q493X	10	192	0.30%	Substitute C>T at 85	TCATTCTGTTCT(C>T)AGTTTTCCTGGATTAT	760/850	304
DI507	10	192	0.50%	Delete 126, 127, 128	ATTAAAGAAAATATcatCTTTGGTGTTTCCTATG	760/850	304
F508C	10	192	rare	Substitute T>G at 131	TAAAGAAAATATCATCT(T>G)TGGTGTTTCCTA	760/850	304
DF508	10	192	67.20%	Delete 129, 130, 131	ATTAAAGAAAATATCATcttTGGTGTTTCCTATG	760/850	304
V520F	10	192	0.20%	Substitute G>T at 166	TAGATACAGAAGC(G>T)TCATCAAAGCATGCC	760/850	304
1717-1G>A	10	95	1.10%	Sub G>A at-1 Ex11	TATTTTTGGTAATA(G>A)GACATCTCCAAGTTT	762/763	233
G542X	11	95	3.40%	Substitute G>T at 40	ACAATATAGTTCTT(G>T)GAGAAGGTGGAAT	762/763	233
S549N	11	95	rare	Substitute G>A at 62	AGGTGGAATCACACTGA(G>A)TGGAGGTCAACG	762/763	233
S549I	11	95	rare	Substitute G>T at 62	AGGTGGAATCACACTGA(G>T)TGGAGGTCAACG	762/763	233
S549R(A>C)	11	95	rare	Substitute A>C at 61	AGGTGGAATCACACTG(A>C)GTGGAGGTCAACG	762/763	233
S549R(T>G)	11	95	0.30%	Substitute T>G at 63	AGGTGGAATCACACTGAG(T>G)GGAGGTCAACG	762/763	233
G551D	11	95	2.40%	Substitute G>A at 68	ATCACACTGAGTGGAG(G>A)TCAACGAGCAAGA	762/763	233
G551S	11	95	rare	Substitute G>A at 67	ATCACACTGAGTGGA(G>A)GTCAACGAGCAAGA	762/763	233
Q552X	11	95	rare	Substitute C>T at 70	ACACTGAGTGGAGGT(C>T)AACGAGCAAGAATT	762/763	233
R553Q	11	95	rare	Substitute G>A at 74	TGAGTGGAGGTCAAC(G>A)AGCAAGAATTTCT	762/763	233
R553X	11	95	1.30%	Substitute C>T at 73	TGAGTGGAGGTCAA(C>T)GAGCAAGAATTTCTTT	762/763	233
A559T	11	95	rare	Substitute G>A at 91	GCAAGAATTTCTTTA(G>A)CAAGGTGAATAAC	762/763	233
R560T	11	95	0.40%	Substitute G>C at 95	AATTTCTTTAGCAA(G>C)GTGAATAACTAA	762/763	233
R560K	11	95	rare	Substitute G>A at 95	GAATTTCTTTAGCAA(G>A)GTGAATAACTAA	762/763	233
1898+1G>A	12	95	0.90%	Sub G>A after last Ex12	GAAATATTTGAAAG(G>A)TATGTTCTTTGAAT	931/932	289
D648V	13	724	Nat Am (63%)	Substitute A>T at 177	AACTCATGGGATGTG(A>T)TTCTTTCGACCAAT	956/884	360
2184 del A	13	724	0.70%	Delete A at 286	GACAGAAACAAAAAAAaCAATCTTTTAAACAGAC	955/884	360
2184 ins A	13	724	rare	Insert A after 286	GACAGAAACAAAAAAAaCAATCTTTTAAACAGAC	955/884	360
2789+5G>A	14b	38	1.10%	Sub G>A 5 one after last	CTCCTTGGAAAGTGA(G>A)TATTCCATGTCCTA	885/886	374
3272-26A>G	17a	228	rare	Sub A>G 26 before 17b	TTTATGTTATTTGCA(A>G)TGTTTTCTATGGAAA	782/901	414
3272-93T>C	17a	228	rare	Sub T>C 93 before 17b	ATTTGTGATATGATTA(T>C)TCTAATTTAGTCTTT	782/901	414
R1066C	17b	228	rare	Substitute C>T at 57	AGGACTATGGACACTT(C>T)GTGCCTTCGGAGCGGC	782/901	414
L1077P	17b	228	rare	Substitute T>C at 91	TTACTTTGAAACTC(T>C)GTTCCACAAAGCTC	782/901	414
Y1092X	17b	228	0.50%	Substitute C>A at 137	CCAACTGGTTCTTGTA(C>A)CTGTCAACACTGCG	782/901	414
M1101K	17b	228	Mut (65%)	Substitute T>A at 163	TGCGCTGGTTCCAAA(T>A)GAGAATAGAAATGAT	782/901	414
R1162X	19	249	0.90%	Substitute C>T at 16	ATGCGATCTGTGAGC(C>T)GAGTCTTTAAGTTC	784/785	356
3659 del C	19	249	0.80%	Delete C at 59	AAGGTAAACCTAcCAAGTCAACCAAACCATACA	784/785	356
3849+4 A>G	19	249	1.00%	Sub A>G 4 after last base	TCCTGGCCAGAGGGTG(A>G)GATTTGAACACT	784/785	356
3849+10kb	19	10kb	1.40%	Sub C>T EcoR1 Fragment	ATAAAATGG(C>T)GAGTAAGACA	792/791	450
W1282R	20	156	rare	Substitute T>C at 127	AATAACTTTGCAACAG(T>C)GGAGGAAAGCCTTT	784/786	351
W1282X	20	156	2.10%	Substitute G>A at 129	AATAACTTTGCAACAGTG(G>A)AGGAAAGCCTTT	784/786	351
3905insT	20	156	2.10%	Insert T at 56	CTTTGTTATCAGCTTTTTTGAGACTACTGAACAC	784/786	351
4005+1 G>A	20	156	Manchester	Sub G>A after Exon 20	AGTGATACCACAG(G>A)TGAGCAAAAGGACTT	784/786	351
N1303K	21	90	1.80%	Substitute C>G at 36	CATTTAGAAAAAA(C>G)TTGGATCCCTATGAAC	788/789	396
N1303H	21	90	rare	Substitute A>C at 34	CATTTAGAAAA(A>C)ACTTGGATCCCTATGAAC		

Table 6

WHAT IS CLAIMED IS:

General tiling claims

1. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least two sets of oligonucleotide probes,

(1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence,

(2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets;

wherein the probes in the first probe set have at least two interrogation positions respectively corresponding to each of two contiguous nucleotides in the reference sequence.

2. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least four sets of oligonucleotide probes,

(1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence,

(2) second, third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence

of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

3. The oligonucleotide array of claim 2, further comprising a fifth probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe from the fifth probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is deleted in the corresponding probe from the fifth probe set.

4. The oligonucleotide array of claim 2, further comprising a sixth probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe from the sixth probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that an additional nucleotide is inserted adjacent to the at least one interrogation position in the corresponding probe from the first probe set.

5. The array of claim 2, wherein the first probe set has at least three interrogation positions respectively corresponding to each of three contiguous nucleotides in a reference sequence.

6. The array of claim 2, wherein the first probe set has at least 50 interrogation positions respectively corresponding to each of 50 contiguous nucleotides in a reference sequence.

7. The array of claim 1 or 2, wherein the first probe set has at least 100 interrogation positions respectively

corresponding to each of 100 contiguous nucleotides in a reference sequence.

8. The oligonucleotide array of claim 1 or 2, wherein the first probe set has an interrogation position corresponding to each of at least 30% of the nucleotides in a reference sequence and the reference sequence comprises at least 100 nucleotides.

9. The oligonucleotide array of claim 8, wherein the first probe set comprises probes which completely span the reference sequence, which probes relative to the reference sequence, overlap one another in sequence.

10. The oligonucleotide array of claim 9, wherein the first probe set has an interrogation position corresponding to each of the nucleotides in the reference sequence.

11. The oligonucleotide array of claim 10, wherein the probes are oligodeoxyribonucleotides.

12. The oligonucleotide array of claim 1 or 2, wherein the array comprises between 100 and 10,000 probes.

13. The oligonucleotide array of claim 1 or 2, wherein the array comprises between 10,000 and 100,000 probes.

14. The oligonucleotide array of claim 1 or 2, wherein the array comprises between 100,000 and 10,000,000 probes.

15. The oligonucleotide array of claim 1 or 2, wherein the probes are linked to the support via a spacer.

16. The oligonucleotide array of claim 1 or 2, wherein the segment in each probe of the first probe set that is exactly complementary to the subsequence of the reference sequence is 9-21 nucleotides.

17. The oligonucleotide array of claim 16, wherein the segment is n nucleotides long, and the subsequence is at least n-2 nucleotides long.

18. The oligonucleotide array of claim 1 or 2, wherein each probe of the first probe set consists of the segment that is exactly complementary to the subsequence of the reference sequence.

19. The oligonucleotide array of claim 1 or 2, wherein the probes in the second, third and fourth probe sets are identical to the corresponding probe from the first probe set except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

20. The array of claim 2, further comprising fifth, sixth and seventh probe sets, wherein:

the segment of each probe in the first set includes at least two interrogation positions each corresponding to a nucleotide in the reference sequence,

the second, third and fourth probe sets, each comprise a corresponding probe for each probe in the first probe set, the corresponding probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes a first interrogation position except that the first interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets;

the fifth, sixth and seventh probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the fifth, sixth and seventh probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes a second interrogation position, except that the second interrogation

position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

21. The array of claim 2, wherein each probe in the first probe set further comprises a second segment of at least three nucleotides exactly complementary to a second subsequence of the reference sequence, and the probes from the second, third and fourth probe sets comprise the corresponding probe from the first probe set or a subsequence thereof comprising the first and second segments except in the at least one interrogation position.

22. The array of claim 2, further comprising:

a fifth probe set comprising at least one probe comprising a segment of at least seven nucleotides exactly complementary to a subsequence of the reference sequence except at one or two positions, the segment including at least one interrogation position corresponding to a nucleotide in the reference sequence not at the one or two positions;

sixth, seventh and eighth probe sets, each comprising a probe for each probe in the fifth probe set, the corresponding probes from the sixth, seventh & eighth probe sets being identical to a sequence comprising the corresponding probe from the fifth probe set or a subsequence of at least nine nucleotides thereof including the at least one interrogation position and the one or two positions, except in the at least one interrogation position, which is occupied by a different nucleotide in each of the four probes.

23. The array of claim 2, wherein the probes are arranged on the substrate so that the first set of probes is arranged in a row across the substrate in an order reflecting the overlap between the probes and the reference sequence, and the additional sets of probes are arranged in columns relative to the probes in said first set, so that probes with the same interrogation position are in the same column and so that each column comprises at least 4 probes.

24. The array of Claim 2, wherein said probes are 12 to 17 nucleotides in length.

25. The array of Claim 2, wherein said probes are 15 nucleotides in length and attached by a covalent linkage to a site on a 3'-end of said probes, and said interrogation position is located at position 7, relative to the 3'-end of said probes.

26. The array of claim 2, further comprises fifth, sixth, seventh and eighth probe sets,

(1) a fifth probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a second reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence,

(2) the sixth, seventh, and eighth probe sets, each comprising a corresponding probe for each probe in the fifth probe set, the probes in the sixth, seventh and eighth probe sets being identical to a sequence comprising the corresponding probe from the fifth probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the fifth, sixth, seventh and eighth probe sets.

27. The array of claim 22, wherein the first, second, third and fourth probe sets have probes of a first length and the fifth, sixth, seventh and eight probe sets have probes of a second length different from the first length.

Tiling for wildtype and mutant reference sequences

28. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least one pair of first and second probe groups, each group comprising a first and second sets of oligonucleotide probes as defined by claim 1;

wherein each probe in the first probe set from the first group is exactly complementary to a subsequence of a first reference sequence and each probe in the first probe set from the second group is exactly complementary to a subsequence from a second reference sequence.

29. The array of claim 28, wherein the second reference sequence is a mutated form of the first reference sequence.

30. The array of claim 28, wherein each group further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the interrogation position, except that the interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

31. The array of claim 30 that comprises at least five pairs of first and second probe groups, wherein the probes in the first probe sets from the first groups of the five pairs are exactly complementary to subsequences from five different respective first reference sequences.

32. The array of claim 30 that comprises at least forty pairs of first and second probe groups, wherein the probes in the first probe sets from the first groups of the forty pairs are exactly complementary to subsequences from forty respective first reference sequences.

Block tiling

33. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least a group of probes comprising:

a wildtype probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a

reference sequence, the segment having at least first and second interrogation positions corresponding to first and second nucleotides in the reference sequence,

a first set of three mutant probes, each identical to a sequence comprising the wildtype probe or a subsequence of at least three nucleotides thereof including the first and second interrogation positions, except in the first interrogation position, which is occupied by a different nucleotide in each of the three mutant probes and the wildtype probe;

a second set of three mutant probes, each identical to a sequence comprising the wildtype probe or a subsequence of at least three nucleotides thereof including the first and second interrogation positions, except in the second interrogation position, which is occupied by a different nucleotide in each of the three mutant probes and the wildtype probe.

34. The array of claim 33, wherein the segment of the wildtype probe comprises 3-20 interrogation positions corresponding to 3-20 respective nucleotides in the reference sequence, and the array comprises 3-20 respective sets of three mutant probes, each of the three probes identical to a sequence comprising the wildtype probe or a subsequence thereof including the 3-20 interrogation positions, except that one of the 3-20 interrogation positions is occupied by a different nucleotide in each of the three mutant probes and the wildtype probes, the one of the 3-20 interrogation positions being different in each of the 3-20 respective sets of three mutant probes.

35. An array of probes immobilized to a solid support comprising two groups of probes, each group as defined by claim 33, a first group comprising a wildtype probe comprising a segment exactly complementary to a subsequence of a first reference sequence and a second group comprising a wildtype probe comprising a segment exactly complementary to a subsequence of a second reference sequence.

36. The array of claim 35, comprising at least 10-100 groups of probes, each comprising a wildtype probe comprising a segment exactly complementary to a subsequence of at least 10-100 respective reference sequences.

Pooled probes

37. A method of comparing a target sequence with a reference sequence, the method comprising:

identifying variants of a reference sequence differing from the reference sequence in at least one nucleotide;

assigning each variant a designation,

providing an array of pools of probes, each pool occupying a separate cell of the array, wherein each pool comprises a probe comprising a segment exactly complementary to each variant sequence assigned a particular designation,

contacting the array with a target sequence comprising a variant of the reference sequence;

determining the relative hybridization intensities of the pools in the array to the target sequence;

determining the target sequence from the relative hybridization intensities of the pools.

38. The method of claim 37, wherein the variants are assigned numbers according to an error code.

39. The method of claim 37, wherein each variant is assigned a designation having at least one digit and at least one value for the digit, and each pool comprise a probe comprising a segment exactly complementary to each variant sequence assigned a particular value in a particular digit.

40. The method of claim 39, wherein the variants are assigned successive numbers in a numbering system of base m having n digits, and the array comprises n x (m-1) pools of probes.

41. The method of claim 40, wherein each pool further comprises a probe comprising a segment exactly complementary to the reference sequence.

Trellis tiling

42. A pooled probe comprising a segment exactly complementary to a subsequence of a reference sequence except at a first interrogation position occupied by a pooled nucleotide N, a second interrogation position occupied by a pooled nucleotide selected from the group of three consisting of (1) M or K, (2) R or Y and (3) S or W, and a third interrogation position occupied by a second pooled nucleotide selected from the group, wherein the pooled nucleotide occupying the second interrogation position comprises a nucleotide complementary to a corresponding nucleotide from the reference sequence when the second pooled probe and reference sequence are maximally aligned, and the pooled nucleotide occupying the third interrogation position comprises a nucleotide complementary to a corresponding nucleotide from the reference sequence when the third pooled probe and the reference sequence are maximally aligned, wherein N is A, C, G or T(U), K is G or T(U), M is A or C, R is A or G, Y is C or T(U), W is A or T(U) and S is G or C.

43. An array of oligonucleotide probes immobilized on solid support, the array comprising:

first, second and third cells respectively occupied by first, second and third pooled probes, each pooled probe comprising a segment exactly complementary to a subsequence of a reference sequence except at a first interrogation position occupied by a pooled nucleotide N, a second interrogation position occupied by a pooled nucleotide selected from the group of three consisting of (1) M or K, (2) R or Y and (3) S or W, and a third interrogation position occupied by a second pooled nucleotide selected from the group, wherein the pooled nucleotide occupying the second interrogation position comprises a nucleotide complementary to a corresponding nucleotide from the reference sequence when the pooled probe

and the reference sequence are maximally aligned, and the pooled nucleotide occupying the third interrogation position comprises a nucleotide complementary to a corresponding nucleotide from the reference sequence when the pooled probe and the reference sequence are maximally aligned;

provided that one of the three interrogation positions in the each of the three pooled probes is aligned with the same corresponding nucleotide in the reference sequence, this interrogation position being occupied by an N in one of the pooled probes, and a different pooled nucleotide in each of the other two pooled probes,

wherein N is A, C, G or T(U), K is G or T(U), M is A or C, R is A or G, Y is C or T(U), W is A or T(U) and S is G or C.

44. The array of claim 43 further comprising:

fourth and fifth cells respectively occupied by fourth and fifth pooled probes, each pooled probe as defined by claim 43,

wherein one of the three interrogation position in the second, third and fourth pooled probes is aligned with the same corresponding nucleotide in the reference sequence, this interrogation position being occupied by an N in one of the pooled probes, and a different pooled nucleotide in each of the other two pooled probes,

wherein one of the three interrogation position in the third, fourth and fifth pooled probes is aligned with the same corresponding nucleotide in the reference sequence, this interrogation position being occupied by an N in one of the pooled probes, and a different pooled nucleotide in each of the other two pooled probes.

45. The array of claim 44, wherein the pooled probes are identical except at the interrogation positions.

46. The array of claim 44, wherein the first, second, third, fourth and fifth pooled probes are exactly complementary to five respective subsequences of the reference

sequences that from each other by increments of one nucleotide.

Bridge tiling

47. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least four probes:

a first probe comprising first and second segments, each of at least three nucleotides and exactly complementary to first and second subsequences of a reference sequences, the segments including at least one interrogation position corresponding to a nucleotide in the reference sequence, wherein either (1) the first and second subsequences are noncontiguous, or (2) the first and second subsequences are contiguous and the first and second segments are inverted relative to the complement of the first and second subsequences in the reference sequence;

second, third and fourth probes, identical to a sequence comprising the first probe or a subsequence thereof comprising at least three nucleotides from each of the first and second segments, except in the at least one interrogation position, which differs in each of the probes.

48. The array of claim 47, wherein the first and second subsequences are separated by one or two nucleotides in the reference sequence.

Two interrogation positions (no wildtype)

49. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least a set of four probes, each of the probes comprising a segment of at least 7 nucleotides that is exactly complementary to a subsequence from a reference sequence, except that the segment may or may not be exactly complementary at two interrogation positions, wherein:

the first interrogation position is occupied by a different nucleotide in each of the four probes,

the second interrogation position is occupied by a different nucleotide in each of the four probes,

in first and second probes, the segment is exactly complementary to the subsequence, except at not more than one of the interrogation positions, and

in third and fourth probes, the segment is exactly complementary to the subsequence, except at both of the interrogation positions.

50. An array of probes immobilized to a support, the array comprising at least 100 sets of 4 probes, each set as defined by claim 49, the probes from the at least 100 sets comprising at least 100 respective segments, the segments having at least 100 respective first and second interrogation positions.

Helper mutations

51. An array of oligonucleotide probes immobilized on a solid support, the array comprising a set of probes comprising:

a first probe comprising a segment of at least 7 nucleotides exactly complementary to a subsequence of a reference sequence except at one or two positions, the segment including an interrogation position not at the one or two positions;

second, third and fourth mutant probes, each identical to a sequence comprising the wildtype probe or a subsequence thereof including the interrogation position and the one or two positions, except in the interrogation position, which is occupied by a different nucleotide in each of the four probes.

Omission of Perfectly Matched Probe

52. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least two sets of oligonucleotide probes,

(1) a first probe set comprising a plurality of probes, each probe comprising a segment exactly complementary to a subsequence of at least 3 nucleotides of a reference sequence except at an interrogation position,

(2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the interrogation position, except that the interrogation position is occupied by a different nucleotide in each of the two corresponding probes and the complement to the reference sequence,

wherein the probes in the first probe set have at least three interrogation positions respectively corresponding to each of three contiguous nucleotides in the reference sequence.

Methods

53. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising:

(a) hybridizing the target nucleic acid to an array of oligonucleotide probes immobilized on a solid support, the array comprising:

(1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence,

(2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets;

wherein, the probes in the first probe set have at least three interrogation positions respectively corresponding

to each of at least three nucleotides in the reference sequence, and

(b) determining which probes, relative to one another, in the array bind specifically to the target nucleic acid, the relative specific binding of the probes indicating whether the target sequence is the same or different from the reference sequence.

54. The method of claim 53, wherein the array further comprises third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

55. The method of claim 54, wherein the target sequence has a substituted nucleotide relative to the reference sequence in at least one undetermined position, and the relative specific binding of the probes indicates the location of the position and the nucleotide occupying the position in the target sequence.

56. The method of claim 54, wherein:

the hybridizing step comprises hybridizing the target nucleic acid and a second target nucleic acid to the array; and

the determining step comprises determining which probes, relative to one another, in the array bind specifically to the target nucleic acid or the second target nucleic acid, the relative specific binding of the probes indicating whether the target sequence is the same or different from the reference sequence and whether the second

target sequence is the same or different from the reference sequence.

57. The method of claim 56, wherein the target sequence has a label and the second target sequence has a second label different from the label.

58. The method of claim 56, wherein undetermined first and second proportions of the first and second target sequences are hybridized to the array and the specific binding indicates the proportions.

59. The method of claim 54, further comprising:

(c) removing the target nucleic acid from the array;

(d) hybridizing a second target nucleic acid to the array;

(e) determining which probes, relative to one another, in the array bind specifically to the second target nucleic acid, the relative specific binding of the probes indicating whether the second target sequence is the same or different from the reference sequence.

60. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising:

hybridizing the target sequence to the array of claim 28;

determining which probes in the first group, relative to one another, hybridize to the target sequence, the relative specific binding of the probes indicating whether the target sequence is the same or different from the first reference sequence;

determining which probes in the second group, relative to one another, hybridize to the target sequence, the relative specific binding of the probes indicating whether the target sequence is the same or different from the second reference sequence.

61. The method of claim 60, wherein the hybridizing step comprising hybridizing the target sequence and a second target sequence to the array, and the relative specific binding of the probes from the first group indicates that the target is identical to the first reference sequence, and the relative specific binding of the probes from the second group indicates that the second target sequence is identical to the second reference sequence.

62. The method of claim 61, wherein the first and second target sequences are heterozygous alleles of a gene.

Comparative hybridization

63. A method of comparing a target nucleic acid with a reference sequence comprising a predetermined sequence of nucleotides, the method comprising:

(a) hybridizing the reference sequence to an array of oligonucleotide probes immobilized on a solid support, the array comprising;

(1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least 3 nucleotides exactly complementary to a subsequence of the reference sequence except in at least one interrogation position;

(2) a second probe set comprising a corresponding probe for each probe in the first probe set, the corresponding probe in the second probe set being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the two corresponding probes from the first and second probe sets; and

(b) determining which probes, relative to one another, in the array bind specifically to the reference sequence;

(c) hybridizing a target sequence to the array;

(d) determining which probes, relative to one another, in the array bind specifically to the target sequence;

wherein the relative specific binding of the probes to the reference and the target sequence indicates whether the reference sequence is the same or different from the target sequence.

64. The method of claim 63, wherein the reference sequence has a first label and the second reference sequence has a second label different from the first label, and steps (a) and (c) are performed simultaneously.

HIV Chip

65. The array of claim 2, wherein the reference sequence is from a human immunodeficiency virus.

66. The array of claim 65, wherein the reference sequence is from a reverse transcriptase gene of the human immunodeficiency virus.

67. The array of claim 66, wherein the reference sequence is from a protease gene of the human immunodeficiency virus.

68. The array of claim 66, wherein the reference sequence is a full-length reverse transcriptase gene.

69. The array of claim 68 comprising at least 3200 oligonucleotide probes.

70. The array of claim 66, wherein the HIV gene is from the BRU HIV strain.

71. The array of claim 66, wherein the HIV gene is from the SF2 HIV strain.

72. The array of claim 28, wherein the reference sequence is from the coding strand of a reverse transcriptase gene of a human immunodeficiency virus and the second reference sequence is from the noncoding strand of the reverse transcriptase gene.

73. The array of claim 28, wherein the first reference sequence is from a reverse transcriptase gene of a human immunodeficiency virus and the second reference sequence comprises a subsequence of the first reference sequence with a substitution of at least one nucleotide.

74. The array of claim 73, wherein the substitution confers drug resistance to a human immunodeficiency virus comprising the second reference sequence.

75. The array of claim 28, wherein the first and second reference sequences are from a reverse transcriptase gene from first and second strains of a human immunodeficiency virus.

76. The array of claim 28, wherein the first reference sequence is from a reverse transcriptase gene of a human immunodeficiency virus and the second reference sequence is from a 16S RNA, or DNA encoding the 16S RNA, from a pathogenic microorganism.

77. The array of claim 28, wherein the first reference sequence is from a reverse transcriptase gene of a human immunodeficiency virus and the second reference sequence is from a protease gene of the human immunodeficiency virus.

78. The method of claim 54, wherein the reference sequence is from a human immunodeficiency virus.

79. The method of claim 78, wherein the reference sequence is from a human immunodeficiency virus and the target sequence is from a second human immunodeficiency virus.

80. The method of claim 79, wherein the target sequence has a substituted nucleotide relative to the reference sequence in at least one undetermined position, and the relative specific binding of the probes indicates the location of the position and the nucleotide occupying the position in the target sequence.

81. The method of claim 80, wherein the target sequence has a substituted nucleotide relative to the reference sequence in at least one position, the substitution conferring drug resistance to the human immunodeficiency virus, and the relative specific binding of the probes reveals the substitution.

82. The method of claim 78, wherein:

the hybridizing step comprises hybridizing the target nucleic acid and a second target nucleic acid, the second target sequence being from a reverse transcriptase gene of a third human immunodeficiency virus, to the array; and

the determining step comprises determining which probes, relative to one another, in the array bind specifically to the target nucleic acid or the second target nucleic acid, the relative specific binding of the probes indicating whether the target sequence is the same or different from the reference sequence and whether the second target sequence is the same or different from the reference sequence.

83. The method of claim 82, wherein the first target sequence has a first label and the second target sequence has a second label different from the first label.

84. The method of claim 82, wherein undetermined first and second proportions of the first and second target sequences are hybridized to the array and the specific binding indicates the proportions.

CFTR Chip

85. The array of claim 2, wherein the reference sequence is from a CFTR gene.

86. The array of claim 85, wherein the reference sequence is exon 10 of a CFTR gene, and said array comprises over 1000 oligonucleotide probes, 10 to 18 nucleotides in length.

87. The array of claim 85, wherein said array comprises a set of probes comprising a specific nucleotide sequence selected from the group of sequences comprising:
3'-TTTATAXTAG;
3'- TTATAGXAGA;
3'- TATAGTXGAA;
3'- ATAGTAXAAA;
3'- TAGTAGXAAC;
3'- AGTAGAXACC;
3'- GTAGAAXCCA;
3'- TAGAAAXCAC; and
3'- AGAAACXACA; wherein each set comprises 4 probes, and X is individually A, G, C, and T for each set.

88. The array of claim 85, wherein said group of sequences comprises:
3'-TTTATAXTAGAAACC;
3'- TTATAGXAGAAACCA;
3'- TATAGTXGAAACCAC;
3'- ATAGTAXAAACCACA;
3'- TAGTAGXAACCACAA;
3'- AGTAGAXACCACAAA;
3'- GTAGAAXCCACAAAG;
3'- TAGAAAXCACAAAGG; and
3'- AGAAACXACAAAGGA; wherein each set comprises 4 probes, and X is individually A, G, C, and T for each set.

89. The array of claim 32, wherein the forty first reference sequences are from a CFTR gene.

90. The array of claim 89, wherein each of the forty first reference sequences includes a site of a mutation and at least one adjacent nucleotide.

91. The array of claim 90, wherein each of the forty first reference sequences comprises at least five contiguous nucleotides from a CFTR gene.

92. The array of claim 89, wherein at least one first reference sequence is a from the coding strand of the cystic fibrosis gene and at least one first reference sequence is from the noncoding strand of the CFTR gene.

93. An array of oligonucleotide probes immobilized on a solid support, the array comprising at least a group of probes comprising:

a wildtype probe exactly complementary to a subsequence of a reference sequence from a cystic fibrosis gene, the segment having at least five interrogation positions corresponding to five contiguous nucleotides in the reference sequence,

a first set of three mutant probes, each identical to the wildtype probe, except in a first of the five interrogation positions, which is occupied by a different nucleotide in each of the three mutant probes and the wildtype probe;

a second set of three mutant probes, each identical to the wildtype probe, except in a second of the five interrogation positions, which is occupied by a different nucleotide in each of the three mutant probes and the wildtype probe;

a third set of three mutant probes, each identical to the wildtype probe, except in a third of the five interrogation positions, which is occupied by a different nucleotide in each of the three mutant probes and the wildtype probe;

a fourth set of three mutant probes, each identical to the wildtype probe, except in a fourth of the five interrogation positions, which is occupied by a different

nucleotide in each of the three mutant probes and the wildtype probe;

a fifth set of three mutant probes, each identical to the wildtype probe, except in a fifth of the five interrogation positions, which is occupied by a different nucleotide in each of the three mutant probes and the wildtype probe.

94. The array of claim 93 comprising first and second groups of probes, each group as defined by claim 93, the first group comprising a wildtype probe exactly complementary to a first reference sequence, and the second group comprising a wildtype probe exactly complementary to a second reference sequence, wherein the second reference sequence is a mutated form of the first reference sequence.

95. The array of claim 94, wherein the first reference sequence is from a CFTR gene and the second reference sequence is a mutated form of the first reference sequence.

96. The method of claim 56, wherein the target sequence and the second target sequence are from heterozygous alleles of a CFTR gene.

P53 Chip

97. The array of claim 2, wherein the reference sequence is a sequence from a p53 gene.

98. The array of claim 2, wherein the reference sequence is from an hMLH1 gene.

99. The array of claim 2, wherein the reference sequence is from an MSH2 gene.

100. The array of claim 28, wherein the reference sequence is from a human P53 gene and the second reference sequence is from an hMLH1 gene.

101. The array of claim 100, further comprising:

ninth, tenth, eleventh and twelfth probe sets,

(1) the ninth probe set comprising a plurality of probes, each probe comprising a segment of at least three nucleotides exactly complementary to a subsequence of a third reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the third reference sequence,

(2) the tenth, eleventh and twelfth probe sets, each comprising a corresponding probe for each probe in the ninth probe set, the probes in the tenth, eleventh and twelfth probe sets being identical to a sequence comprising the corresponding probe from the ninth probe set or a subsequence of at least three nucleotides thereof that includes the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the ninth, tenth, eleventh and twelfth probe sets.

102. The array of claim 97, wherein the first probe set has at least 60 interrogation positions corresponding to at 60 contiguous nucleotides from exon 6.

103. The array of claim 98, wherein the reference sequence is exon 5 of a p53 gene, the probes are 17 nucleotides long, and the first set of probes is exactly complementary to the reference sequence, and the at least one interrogation position is at position 7, relative to a 3'-end of each probe, which 3'-end is covalently attached to the substrate.

Mitochondrial Chip

104. The array of claim 2, wherein the reference sequence is from a mitochondrial genome.

105. The array of claim 104, wherein said reference sequence is a sequence of a D-loop region.

106. The array of claim 105, wherein D-loop region is full-length.

107. The array of claim 104, wherein said reference sequence is at least 90% of a full-length mitochondrial genome.

108. The array of claim 104, wherein the reference sequence is bounded by positions 16280 to 356 of the mitochondrial genome.

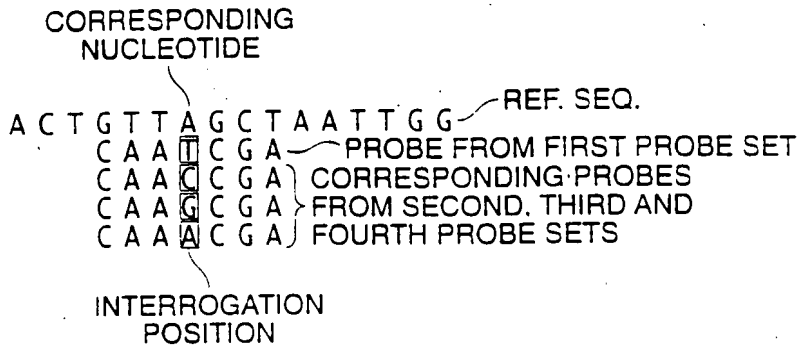

CORRESPONDING NUCLEOTIDE
A C T G T T A G C T A A T T G G
REF. SEQ.
C A A T C G A
PROBE FROM FIRST PROBE SET
C A A C C G A
C A A G C G A
C A A A C G A
CORRESPONDING PROBES FROM SECOND, THIRD AND FOURTH PROBE SETS
INTERROGATION POSITION

FIG. 1

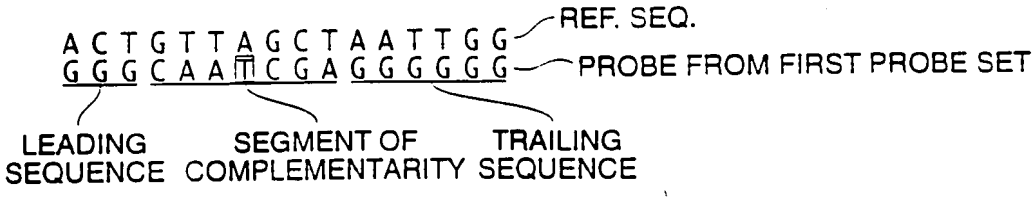

A C T G T T A G C T A A T T G G
REF. SEQ.
G G G C A A T C G A G G G G G G
PROBE FROM FIRST PROBE SET
LEADING SEQUENCE
SEGMENT OF COMPLEMENTARITY
TRAILING SEQUENCE

FIG. 2

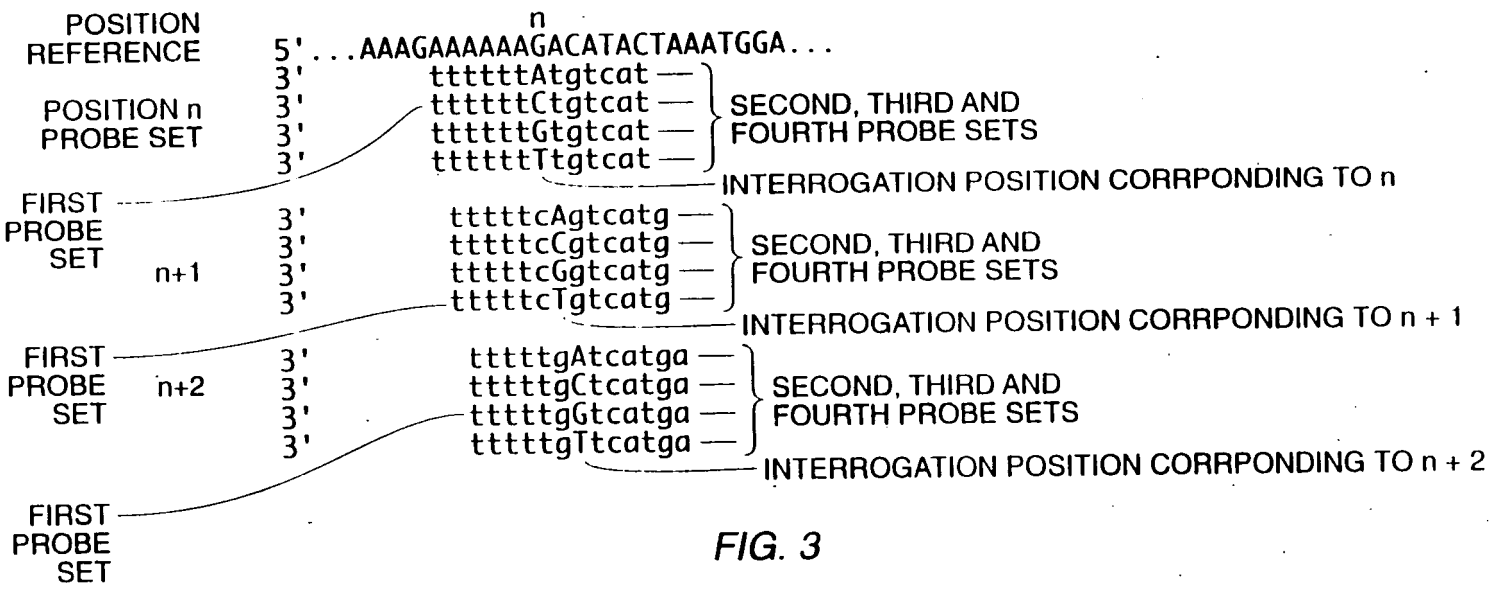


FIG. 3

XX/XX

XX/XX

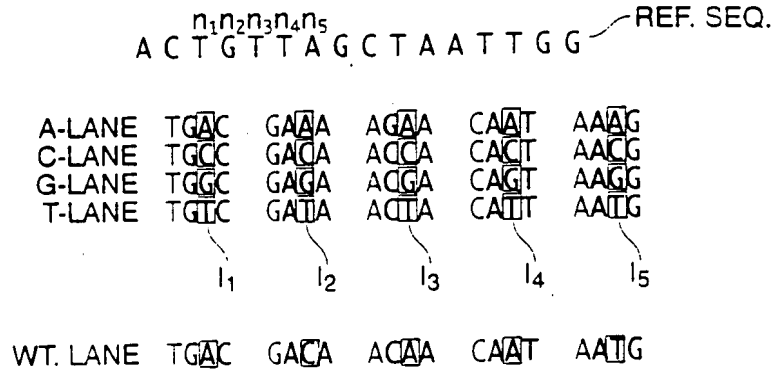
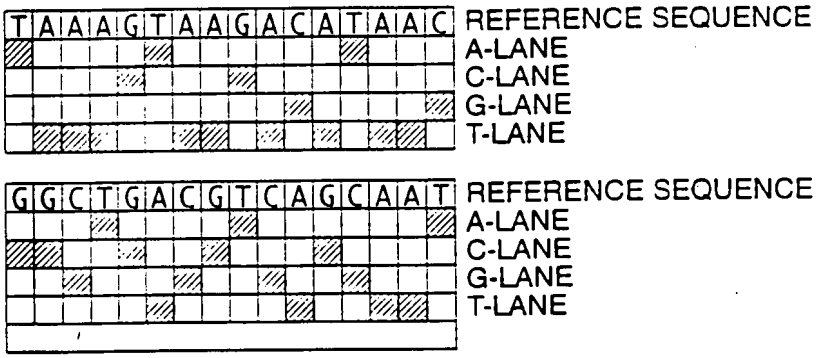


FIG. 4



3'-CCGACTACAGTCGTT
3'-CCGACTCCAGTCGTT
3'-CCGACTGCAGTCGTT
3'-CCGACTTCAGTCGTT

FIG. 5

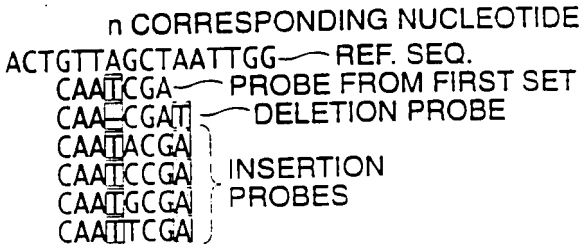

n CORRESPONDING NUCLEOTIDE
ACTGTTAGCTAATTGG— REF. SEQ.
CAATCGA— PROBE FROM FIRST SET
CAA_CGAT — DELETION PROBE
CAATACGA
CAATCCGA INSERTION
CAATGCGA PROBES
CAATTCGA

FIG. 6

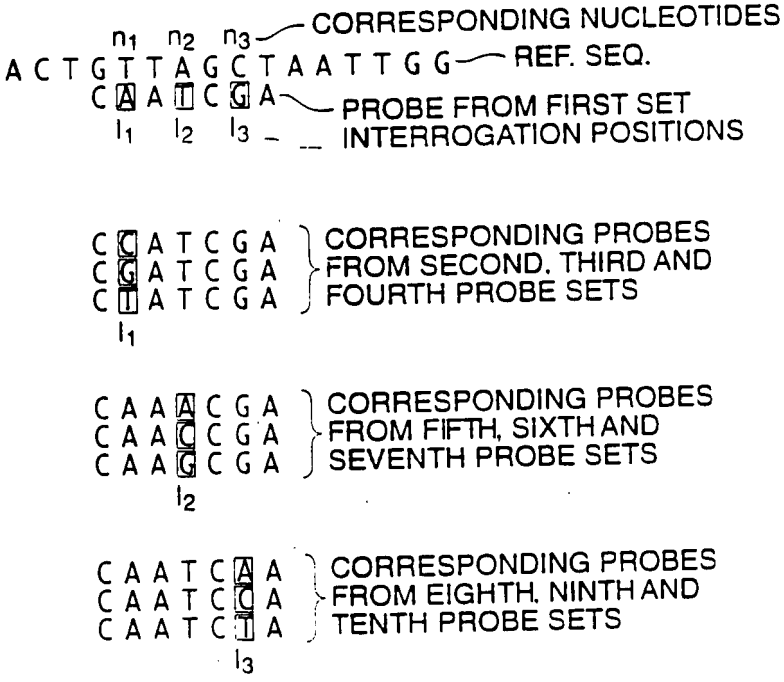

n1 n2 n3 — CORRESPONDING NUCLEOTIDES
A C T G T T A G C T A A T T G G — REF. SEQ.
C A A T C G A — PROBE FROM FIRST SET
I1 I2 I3 INTERROGATION POSITIONS
C C A T C G A
C G A T C G A CORRESPONDING PROBES FROM SECOND, THIRD AND FOURTH PROBE SETS
C T A T C G A
I1
C A A A C G A
C A A C C G A CORRESPONDING PROBES FROM FIFTH, SIXTH AND SEVENTH PROBE SETS
C A A G C G A
I2
C A A T C A A
C A A T C C A CORRESPONDING PROBES FROM EIGHTH, NINTH AND TENTH PROBE SETS
C A A T C T A
I3

FIG. 7

XX/XX

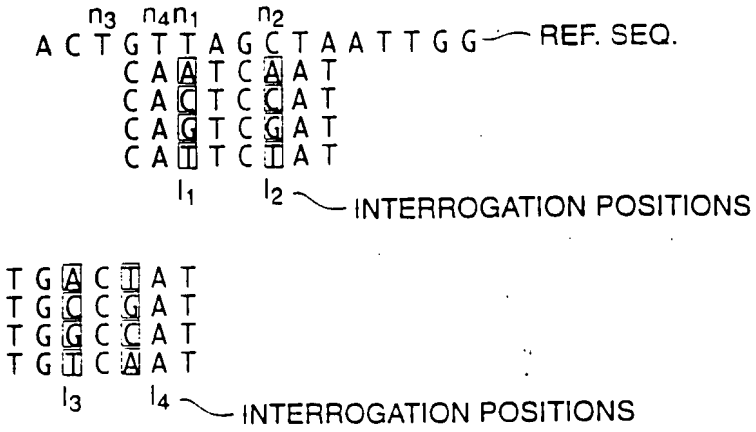


FIG. 8

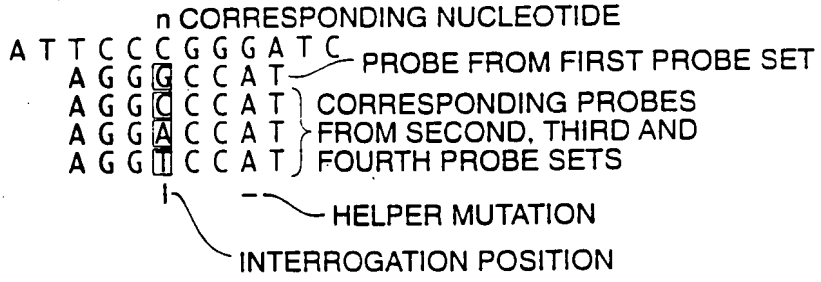


FIG. 9

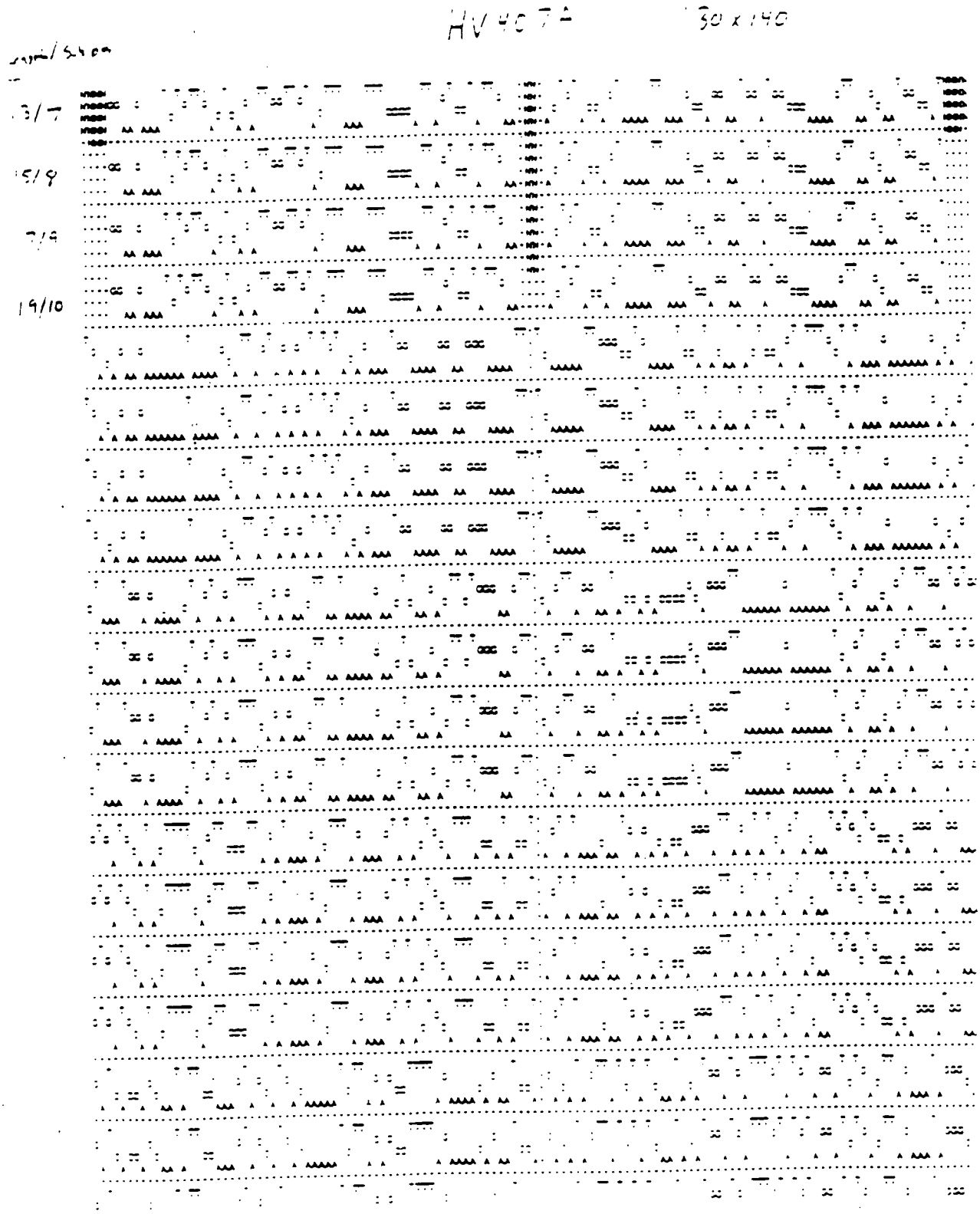


Fig. 10
Page 1 of 2

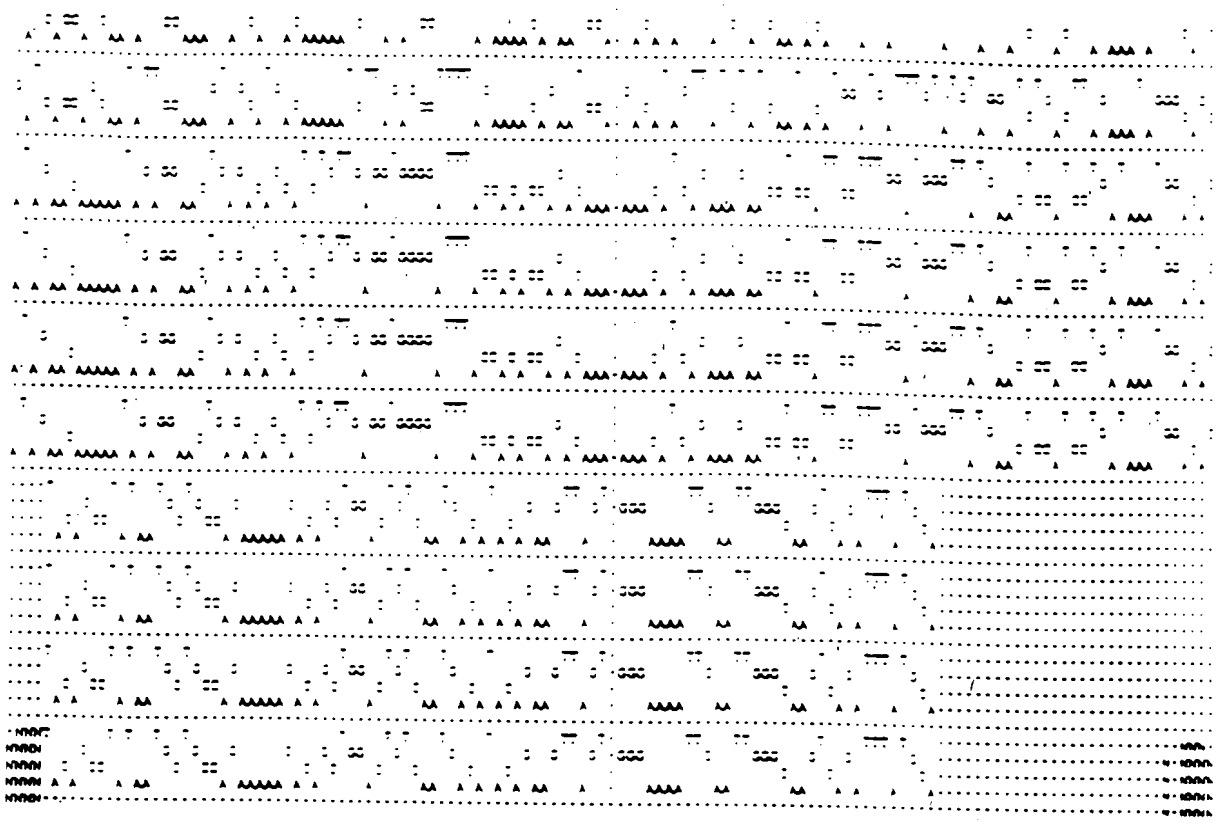
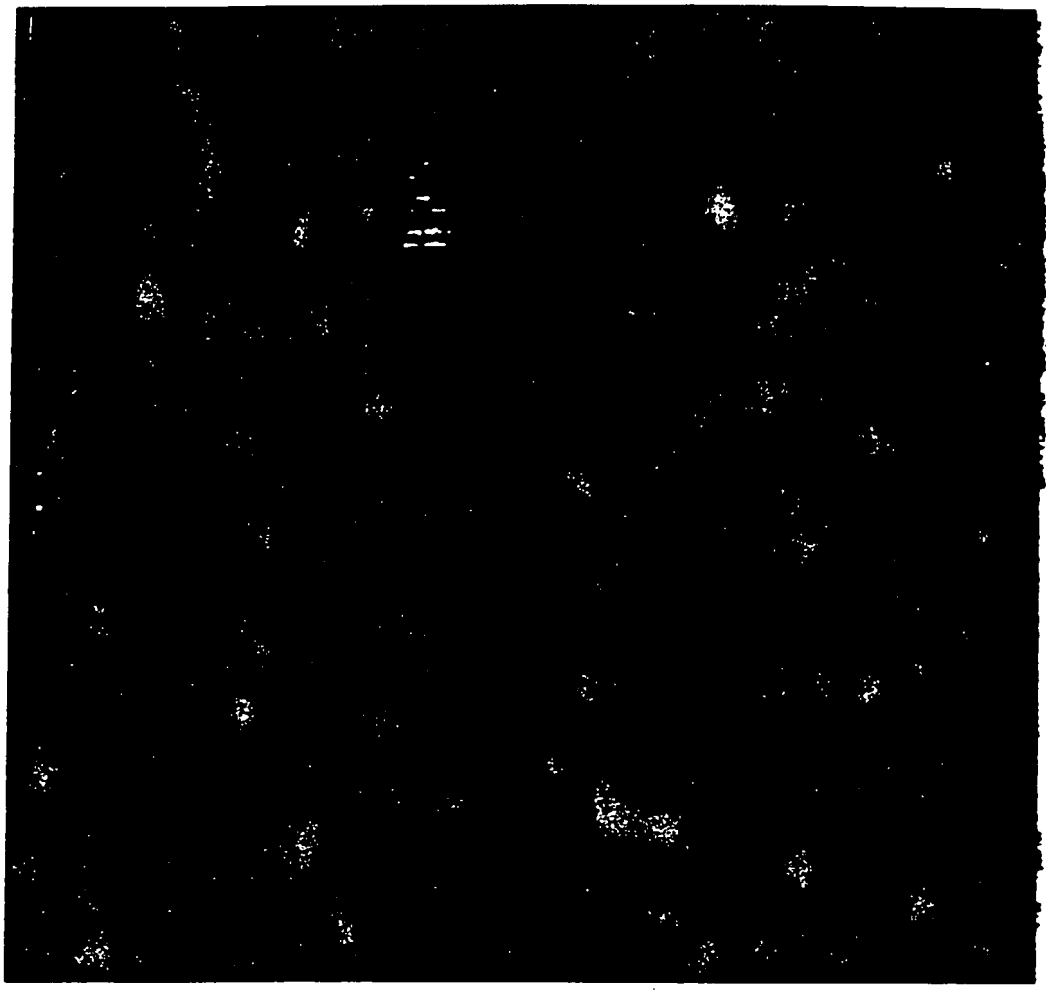


Fig. 10
Page 2 of 2



MCC7360:
=407 water chip hyondized with fragmented pfol 19 RNA

Fig. 11

Figure 12
(Page 1 of 2)

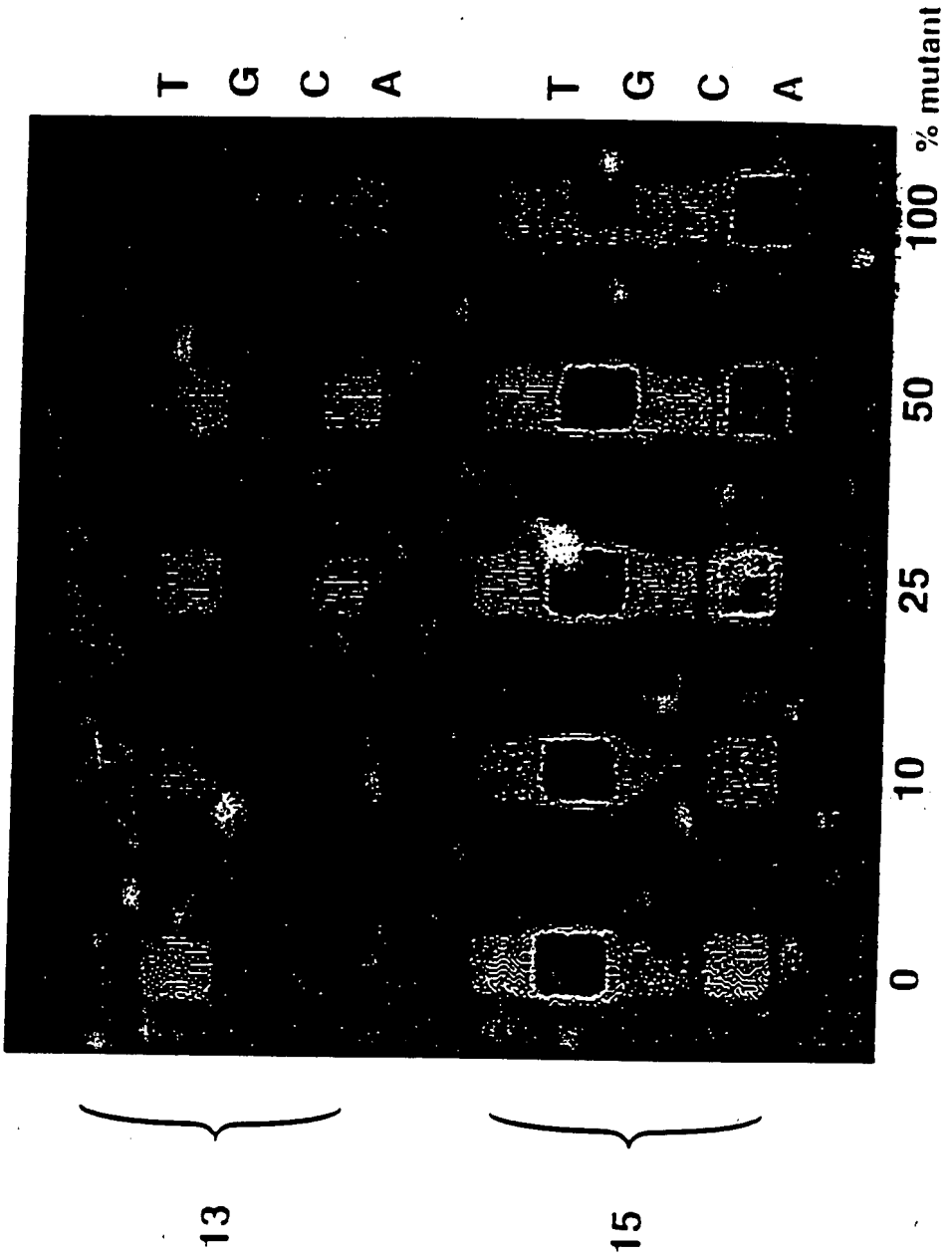
Figure 12
(Page 2 of 2)

```
5'Fluorescein-AAAGAAAAAAGACAGTACTAAATGGAGAAAAT wildtype
PROBE 3'          ttttttt•tgtcat          13mers
PROBE 3'        ctttttttt•tgtcatg         15mers
PROBE 3'      tctttttttt•tgtcatga         17mers
PROBE 3'    ttctttttttt•tgtcatgat         19mers
5'Fluorescein-AAAGAAAAAAACAGTACTAAATGGAGAAAAT mutant
```

Fig. 13

- 57 -

Fig. 14



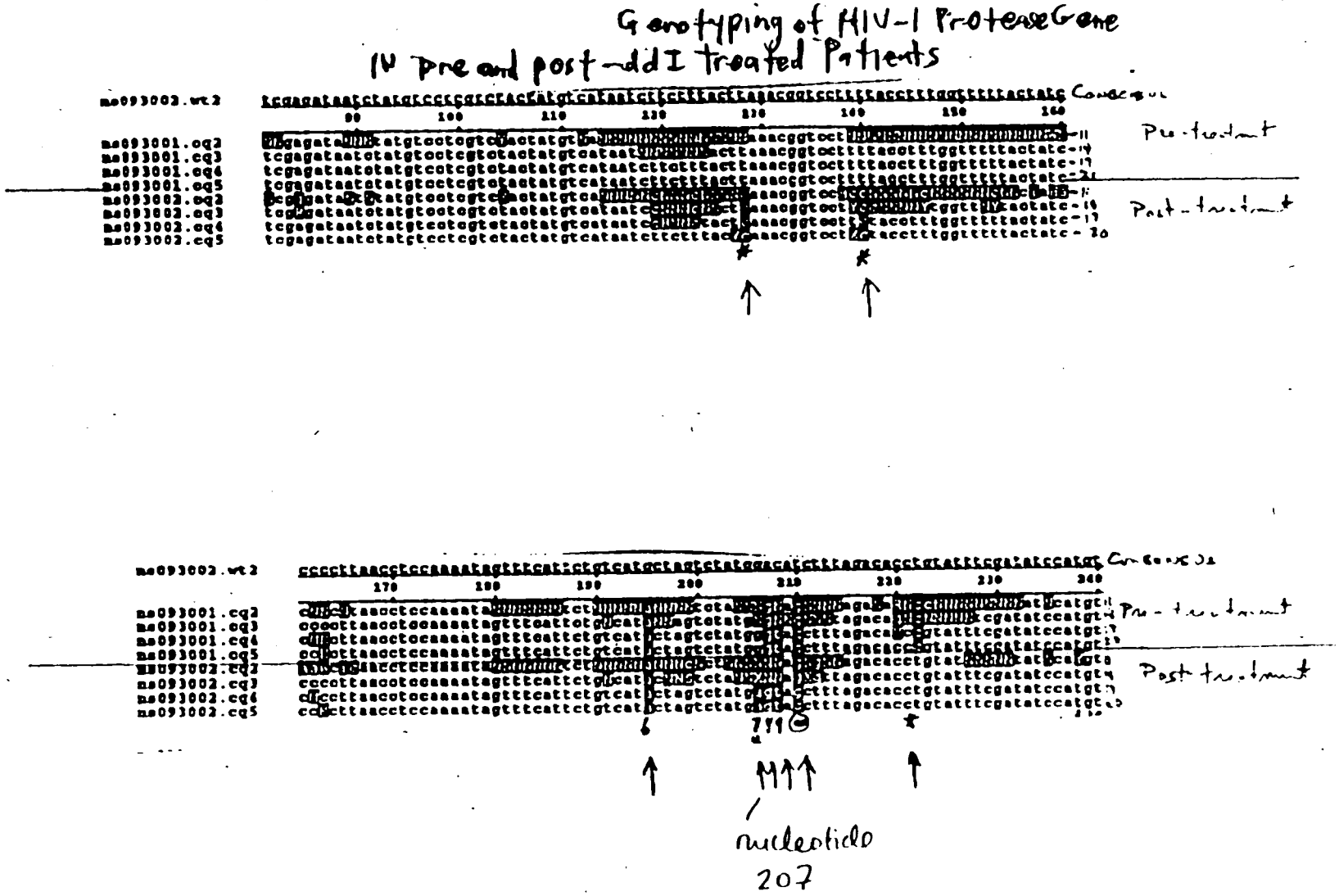
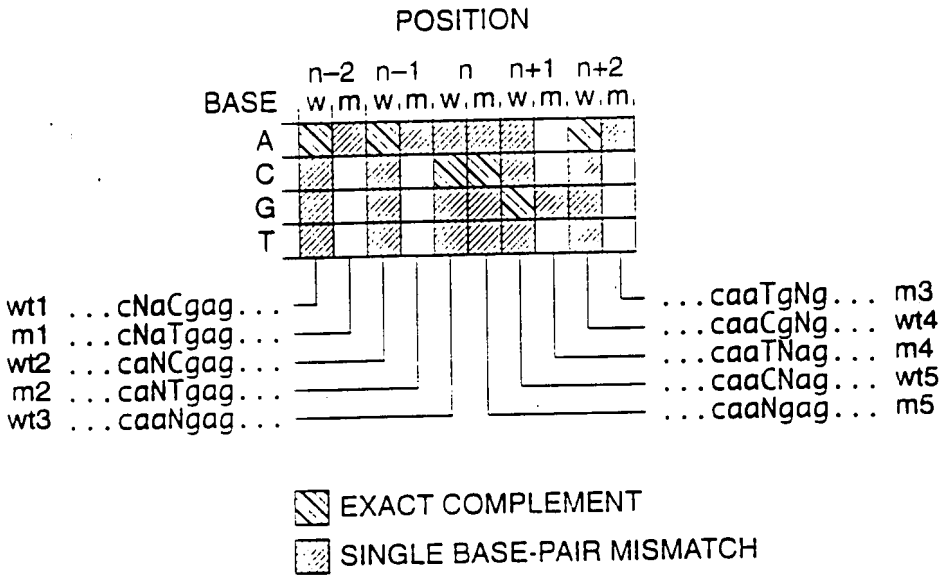

Genotyping of HIV-1 Protease Gene
IN pre and post-ddI treated Patients
ms093002.wt2
ms093001.cq2
ms093001.cq3
ms093001.cq4
ms093001.cq5
ms093002.cq2
ms093002.cq3
ms093002.cq4
ms093002.cq5
Pre-treatment
Post-treatment
nucleotide
207

Fig. 15

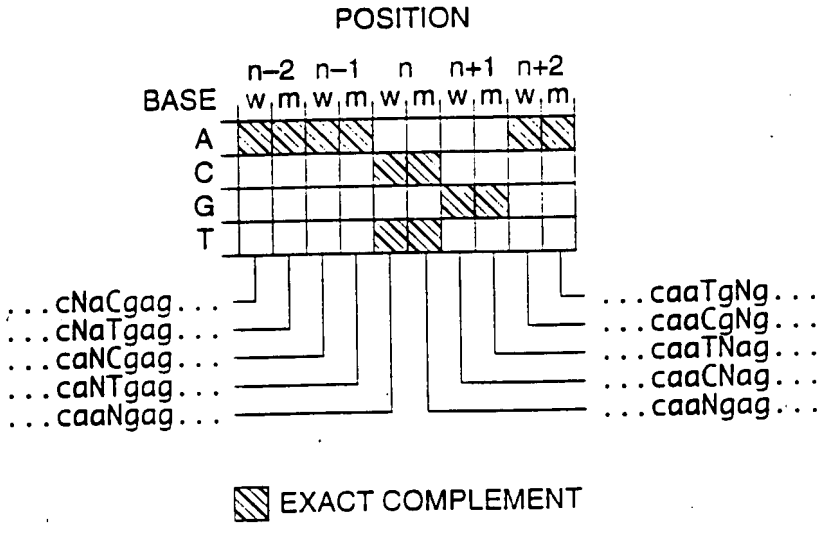
XX/XX



WILD-TYPE SEQUENCE: 5'=AGGTCAACGAGCAA=3'

MUTANT SEQUENCE: 5'=AGGTCAATGAGCAA=3'

FIG. 16



WILD-TYPE SEQUENCE: 5'=AGGTCAACGAGCAA=3'

MUTANT SEQUENCE: 5'=AGGTCAATGAGCAA=3'

FIG. 17

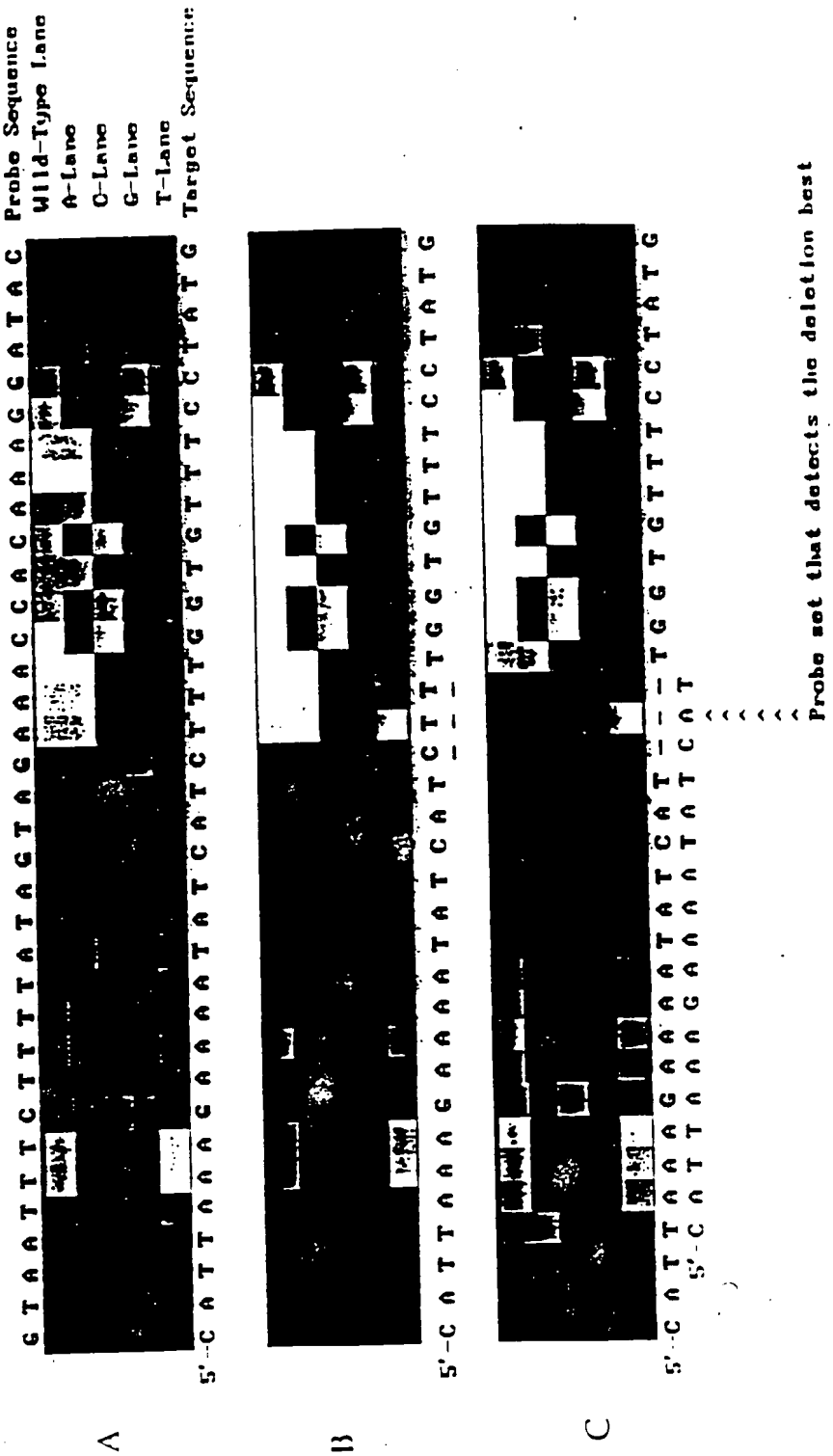

Probe Sequence
Wild-Type Lane
A-Lane
C-Lane
G-Lane
T-Lane
Target Sequence
5'-CATTAAAGAAAATATCATCTTTGGTGTTTCCTATG
5'-CATTAAAGAAAATATCATCTTTGGTGTTTCCTATG
5'-CATTAAAGAAAATATCAT---TGGTGTTTCCTATG
Probe set that detects the deletion best
A
B
C

Fig. 18

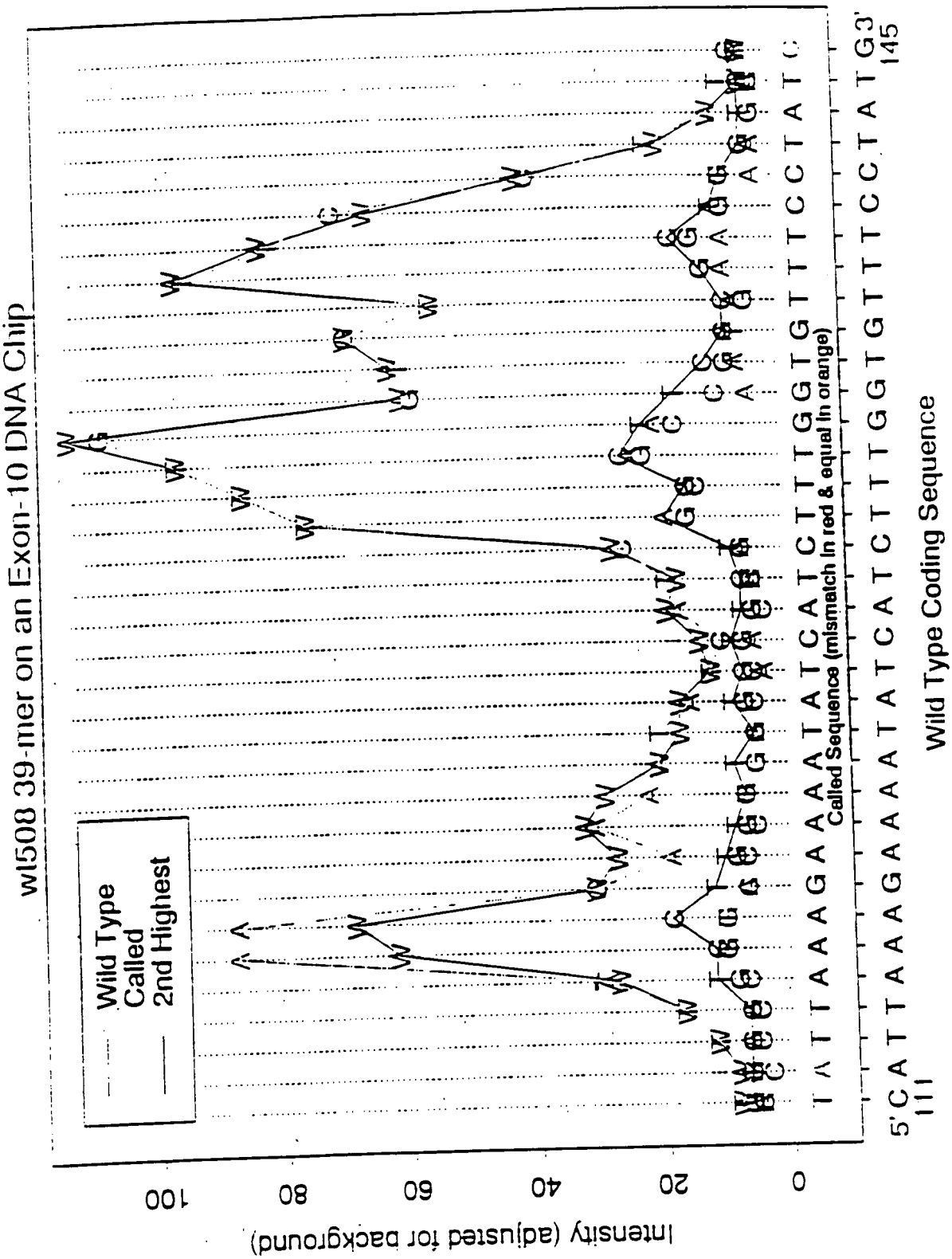


Fig. 19
Page 1 of 3

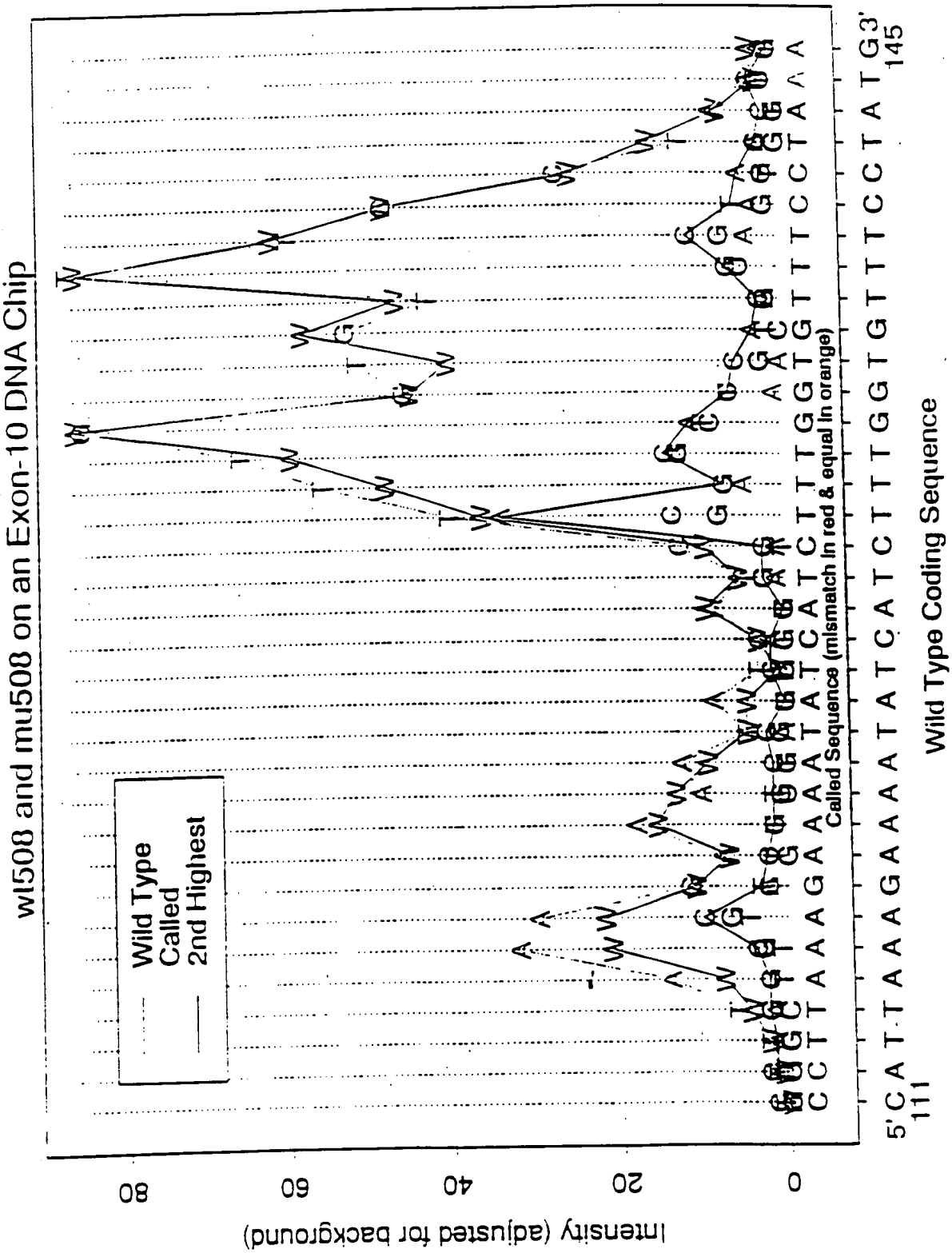


Fig. 19
Page 2 of 3

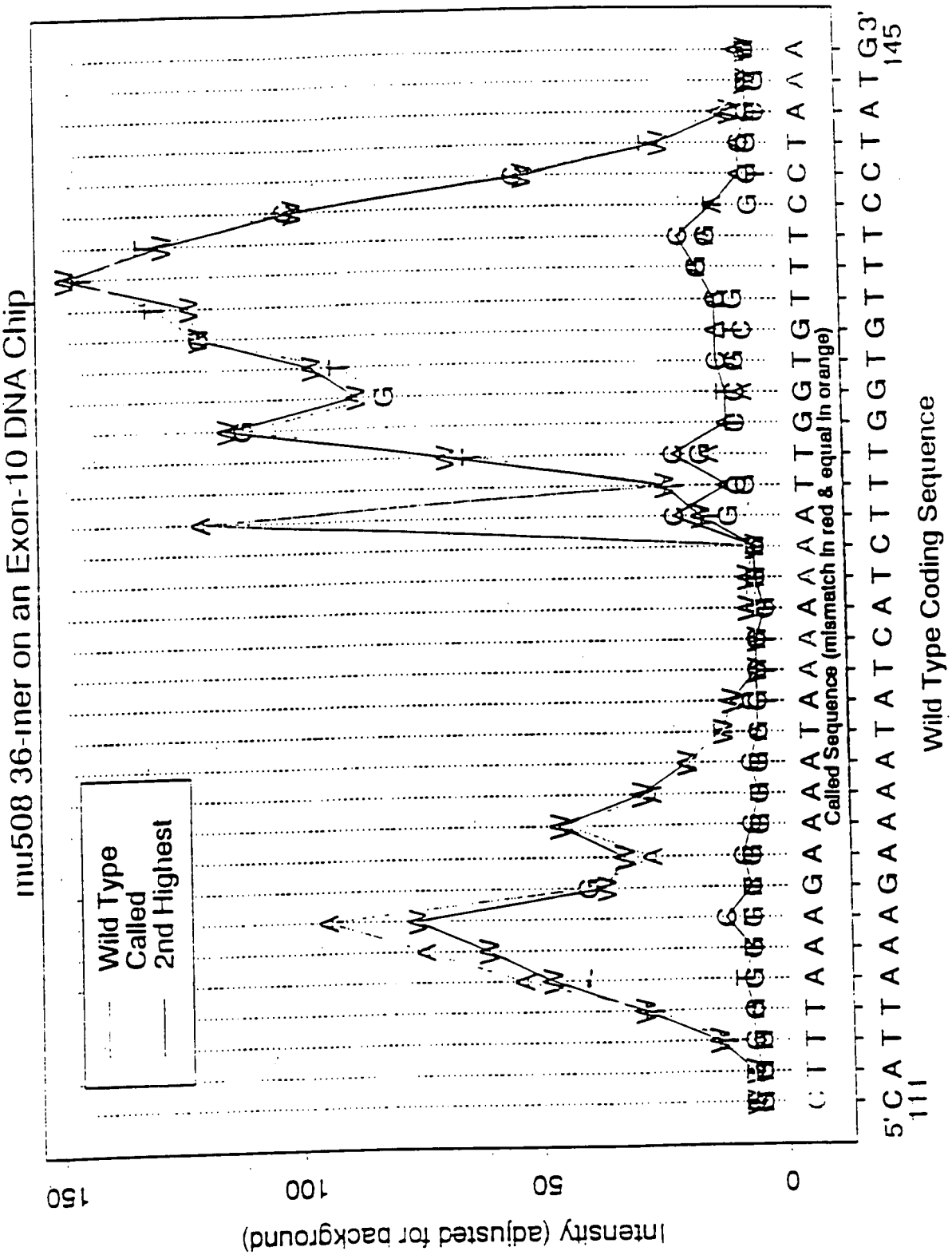


Fig. 19
Page 3 of 3

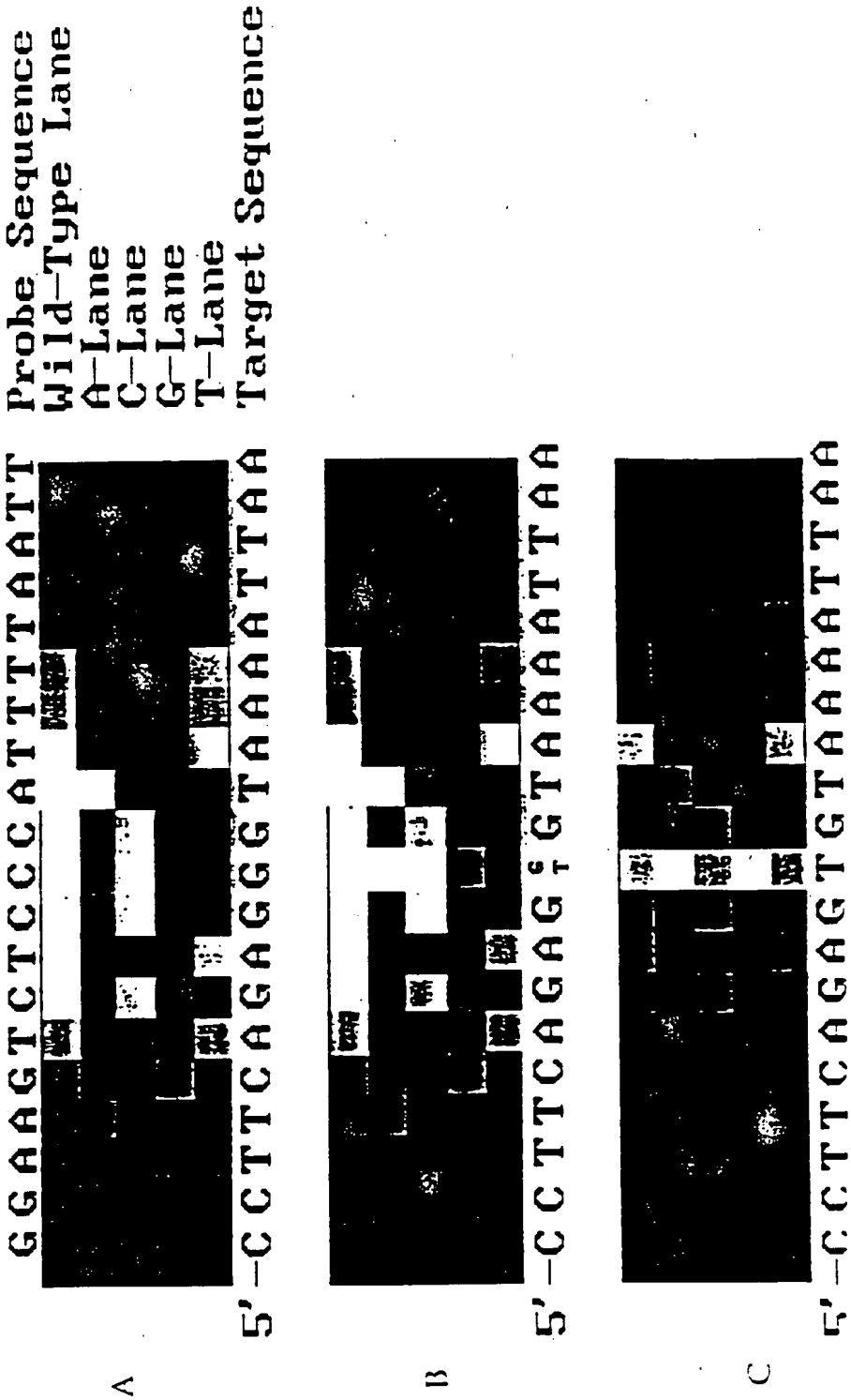

Probe Sequence
Wild-Type Lane
A-Lane
C-Lane
G-Lane
T-Lane
Target Sequence
GGAAGTCTCCCATTTTAATT
5'-CCTTCAGAGGGTAAAATTAA
A
5'-CCTTCAGAG T GTAAAATTAA
B
5'-CCTTCAGAGTGTAAAATTAA
C

Fig. 20

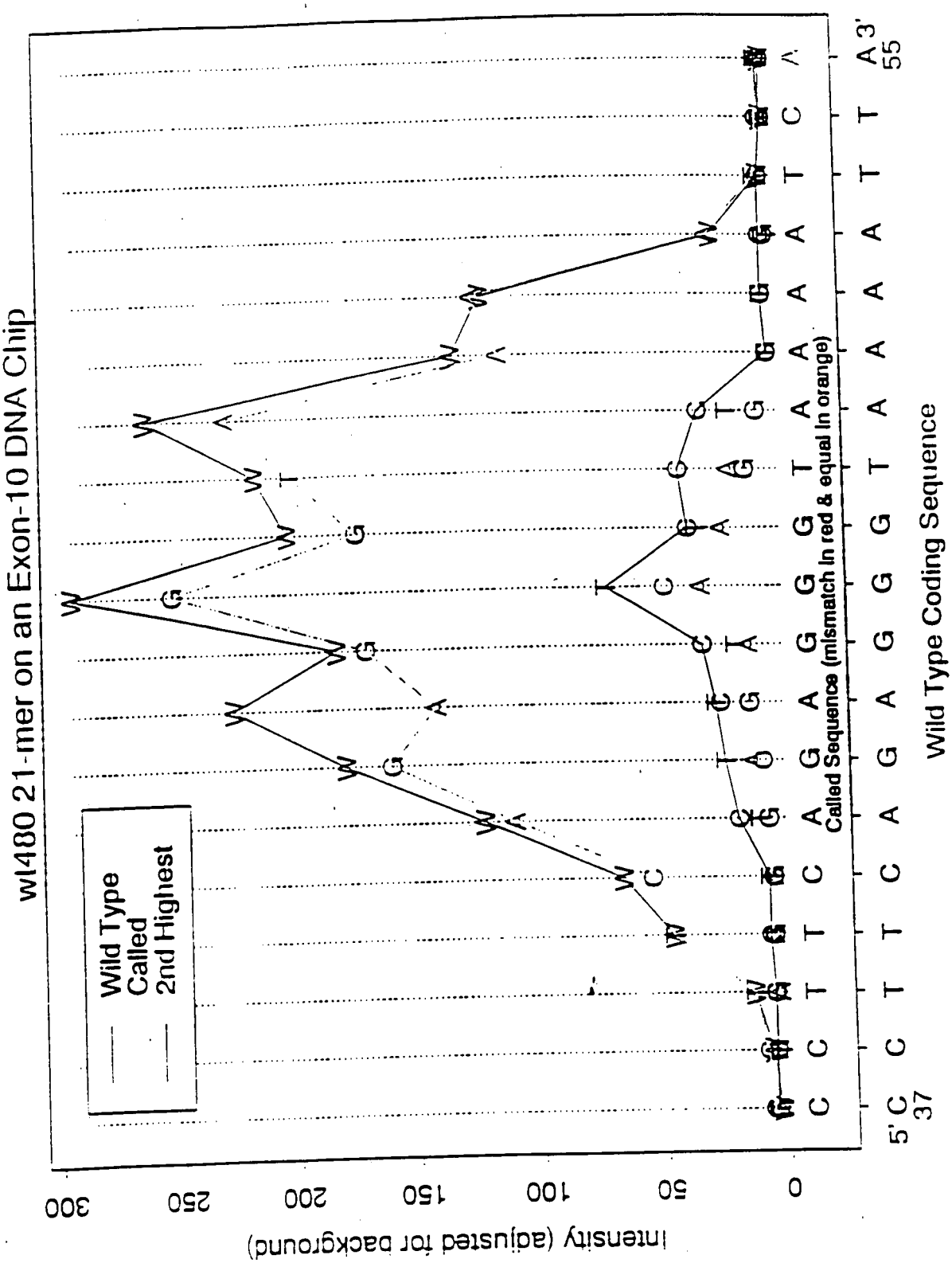


Fig. 21
Page 1 of 3

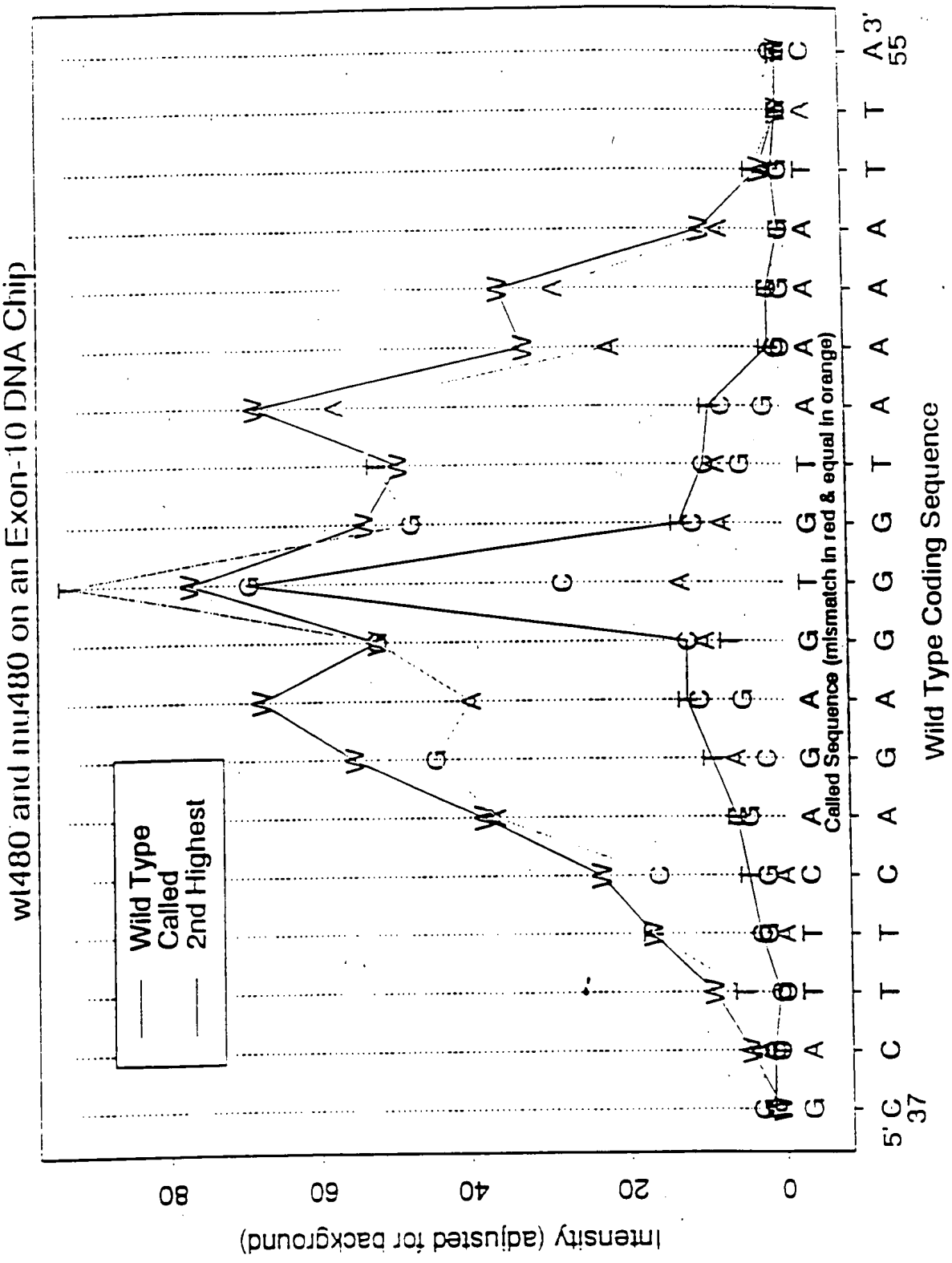


Fig. 21
Page 2 of 3

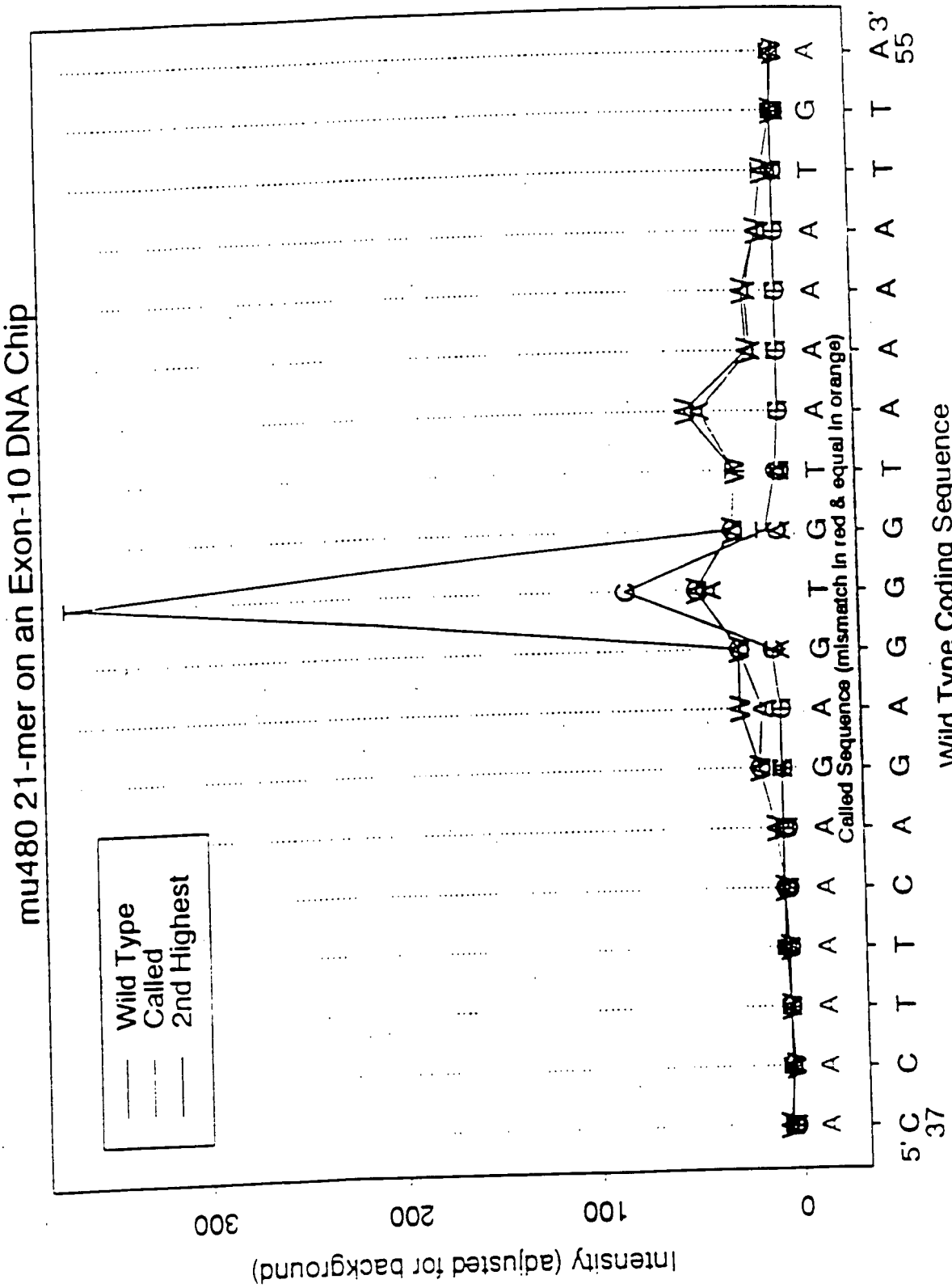


Fig. 21
Page 3 of 3

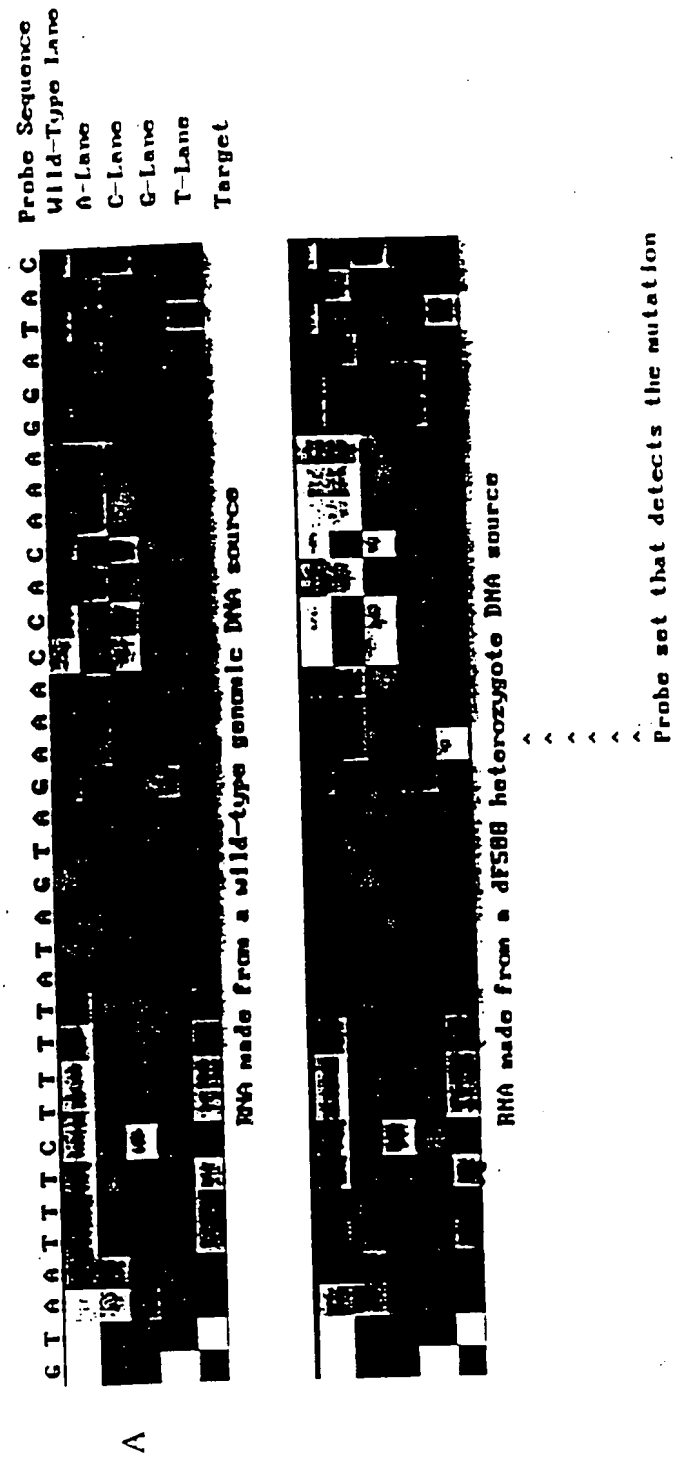

Probe Sequence
Wild-Type Lane
A-Lane
C-Lane
G-Lane
T-Lane
Target
GTAATTTCTTTATAGTAGAAAACCACAAAGGATAC
DNA made from a wild-type genomic DNA source
RNA made from a dF508 heterozygote DNA source
Probe set that detects the mutation
A

Fig. 22

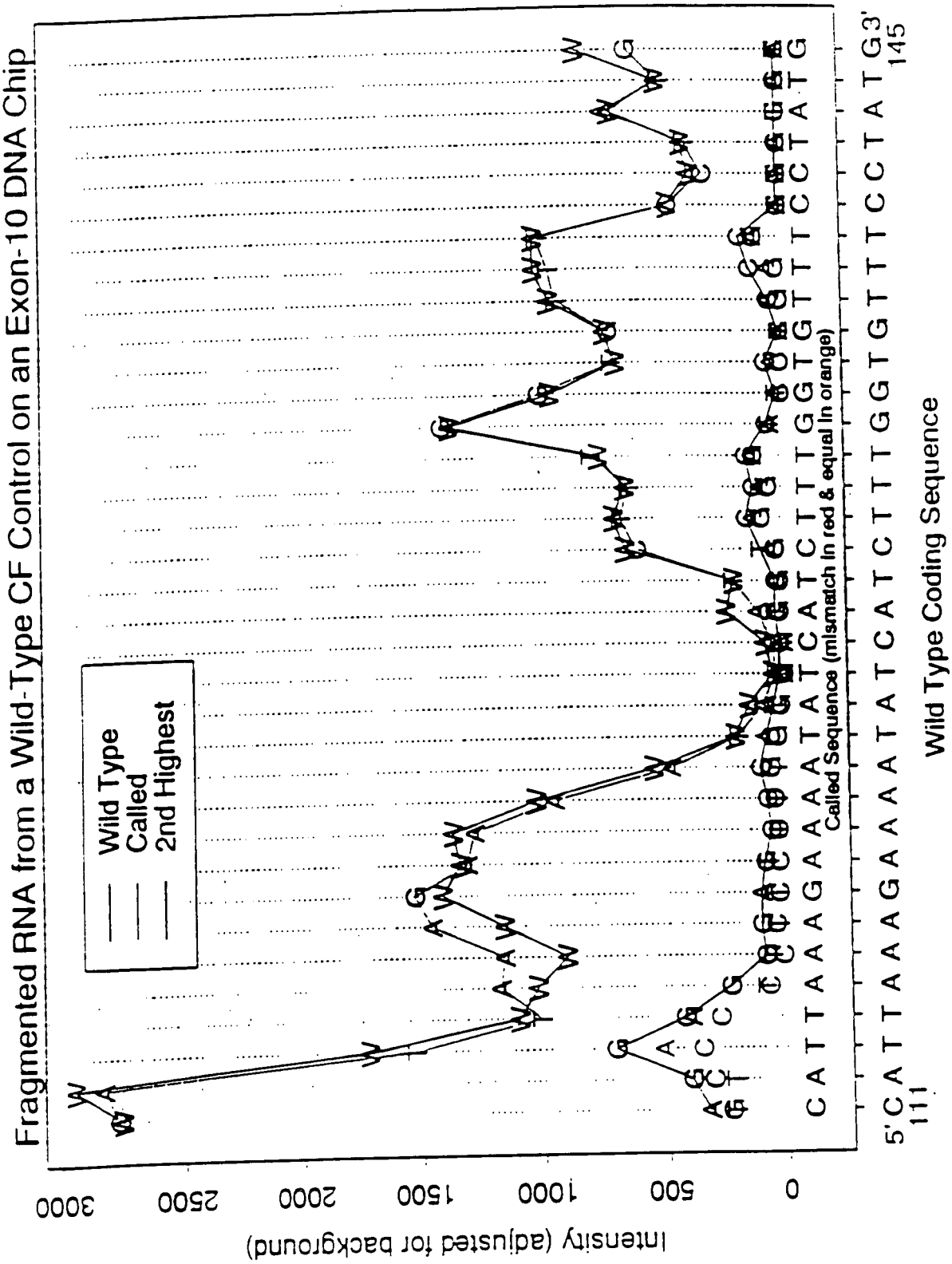


Fig. 23
Page 1 of 2

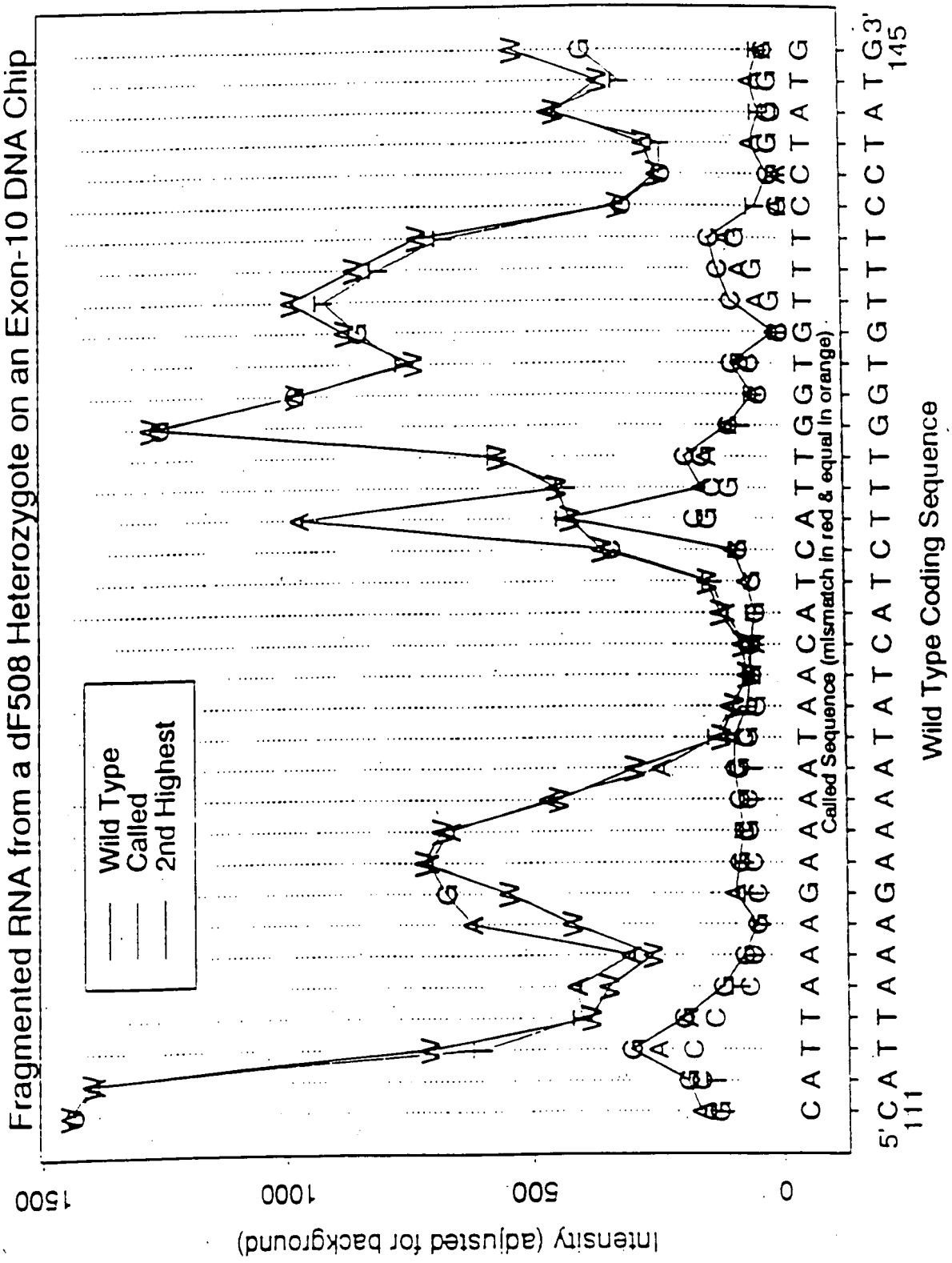

Fragmented RNA from a dF508 Heterozygote on an Exon-10 DNA Chip
Wild Type
Called
2nd Highest
Intensity (adjusted for background)
0
500
1000
1500
5'CATTTAAAGAAAATATCATCTTTGGTGTTTCCTATG3'
111
145
CATTTAAAGAAAATAACATCATTGGTGTTTCCTATG
Called Sequence (mismatch in red & equal in orange)
Wild Type Coding Sequence

Fig. 23

23.67

A B

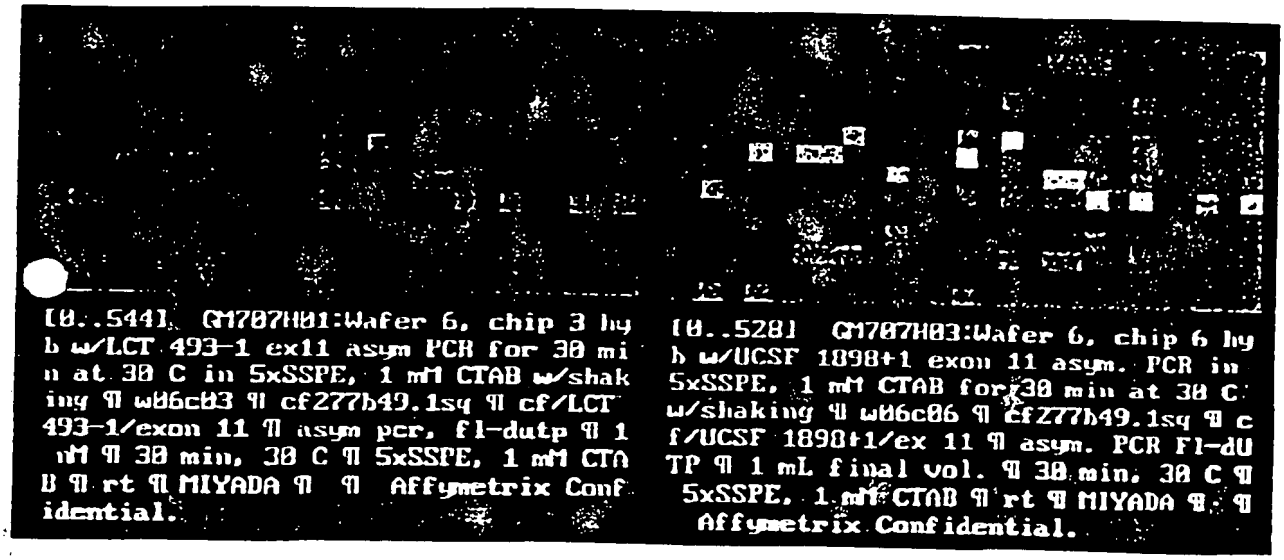


Fig. 24

A

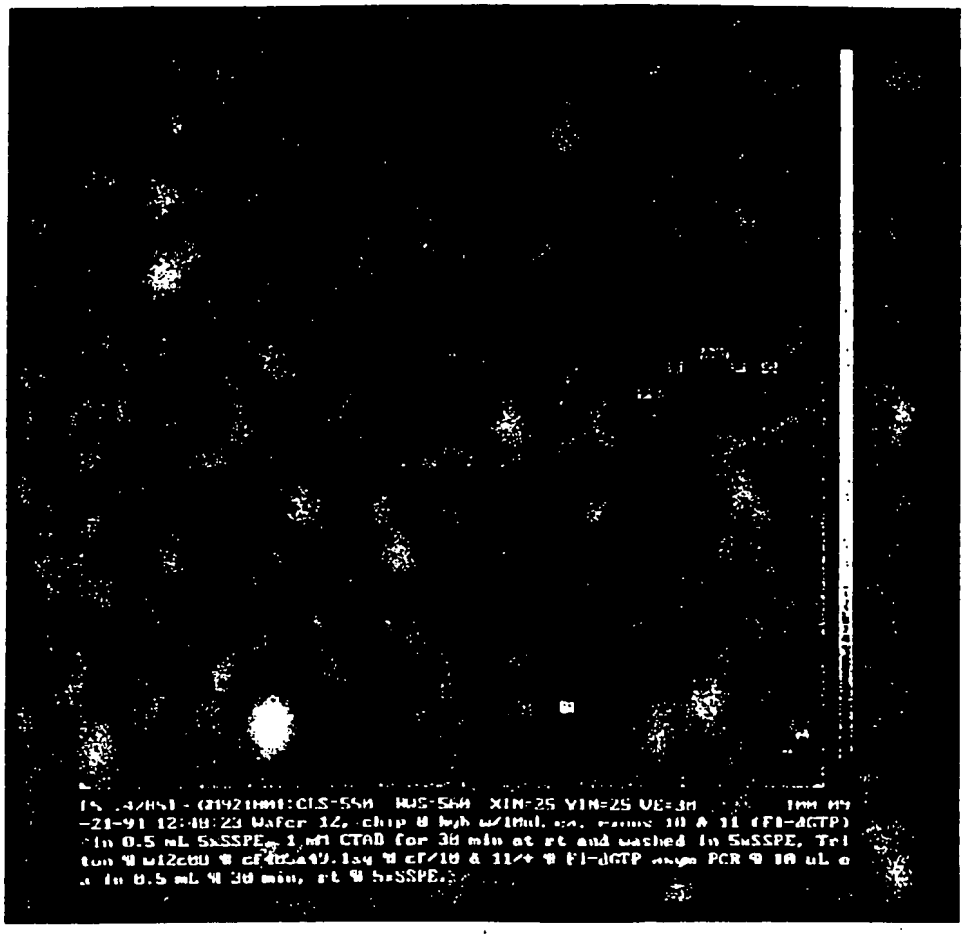


Fig. 25
Page 1 of 2

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B

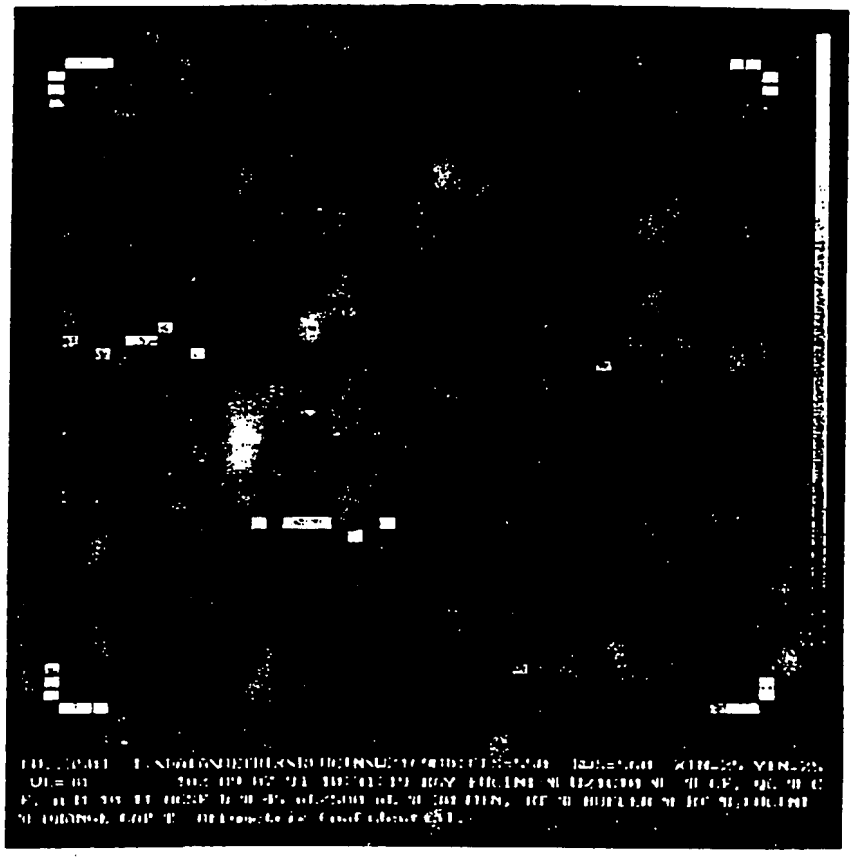


Fig. 25
Page 2 of 2

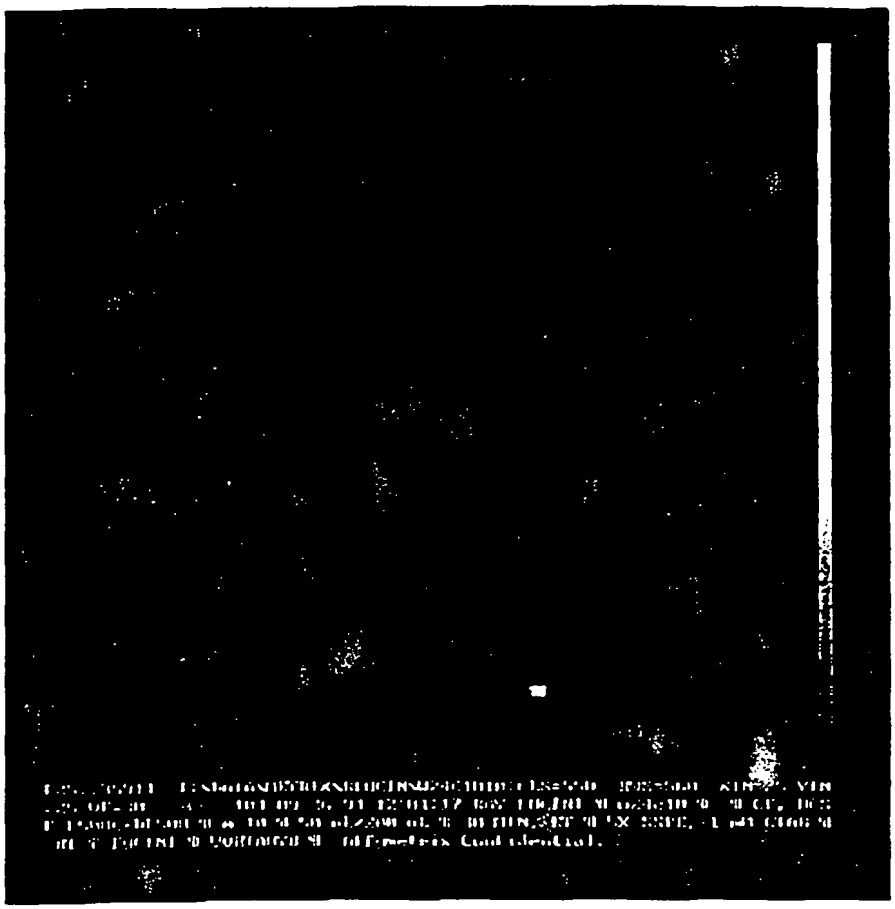


Fig. 26

Fig. 27

P53 EXON 6 CODON 192 REGION: 12MER PROBES

	G	A	T	G	C	T	G	A	G	G	A	G
0.						C	C	C	C	C	C	T
1.						A	T	T	T	T	T	G
2.						C	C	C	C	C	C	G
3.						C	C	C	C	C	C	G
4.						C	C	C	C	C	C	G
5.						C	C	C	C	C	C	G
6.						A	A	A	A	A	A	A
7.						G	G	G	G	G	G	G
8.						C	C	C	C	C	C	C
9.						A	A	A	A	A	A	A
10.						T	T	T	T	T	T	T
11.						C	C	C	C	C	C	C
12.						C	C	C	C	C	C	T

P53 EXON 6 CODON 192 REGION: 10MER PROBES

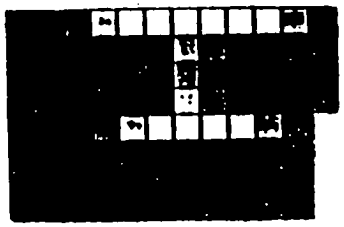
	G	A	T	G	C	T	G	A	G	G
0.							T	C	C	C
1.							C	T	C	C
2.							A	C	T	C
3.							G	A	C	T
4.							C	G	A	C
5.							A	C	G	A
6.							-	T	A	C
7.								C	T	A
8.								T	C	T
9.								T	T	C
10.								A	T	T
11.								T	A	T

Fig. 28

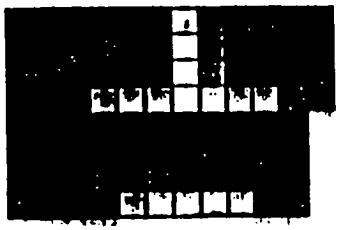
Detection of 12-mer One-Base Sustitution P53 Targets

Fig. 29

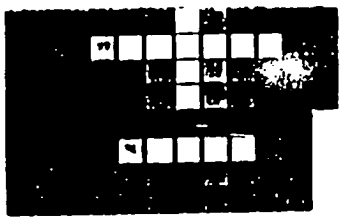
WT ("G" Substitution) Target 12-mer



"A Substitution 12-mer Target



"T" Substitution Target 12-mer



"C"Substitution Target 12-mer

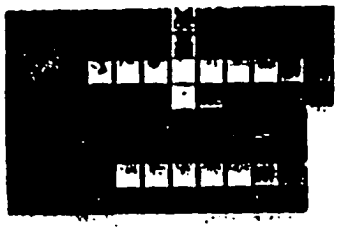


Fig. 31

4:1 Mixture of WT and "A" Substitution 12-mer Targets

Figs. 29 and 31

P53 EXON 6 CODON 192 REGION

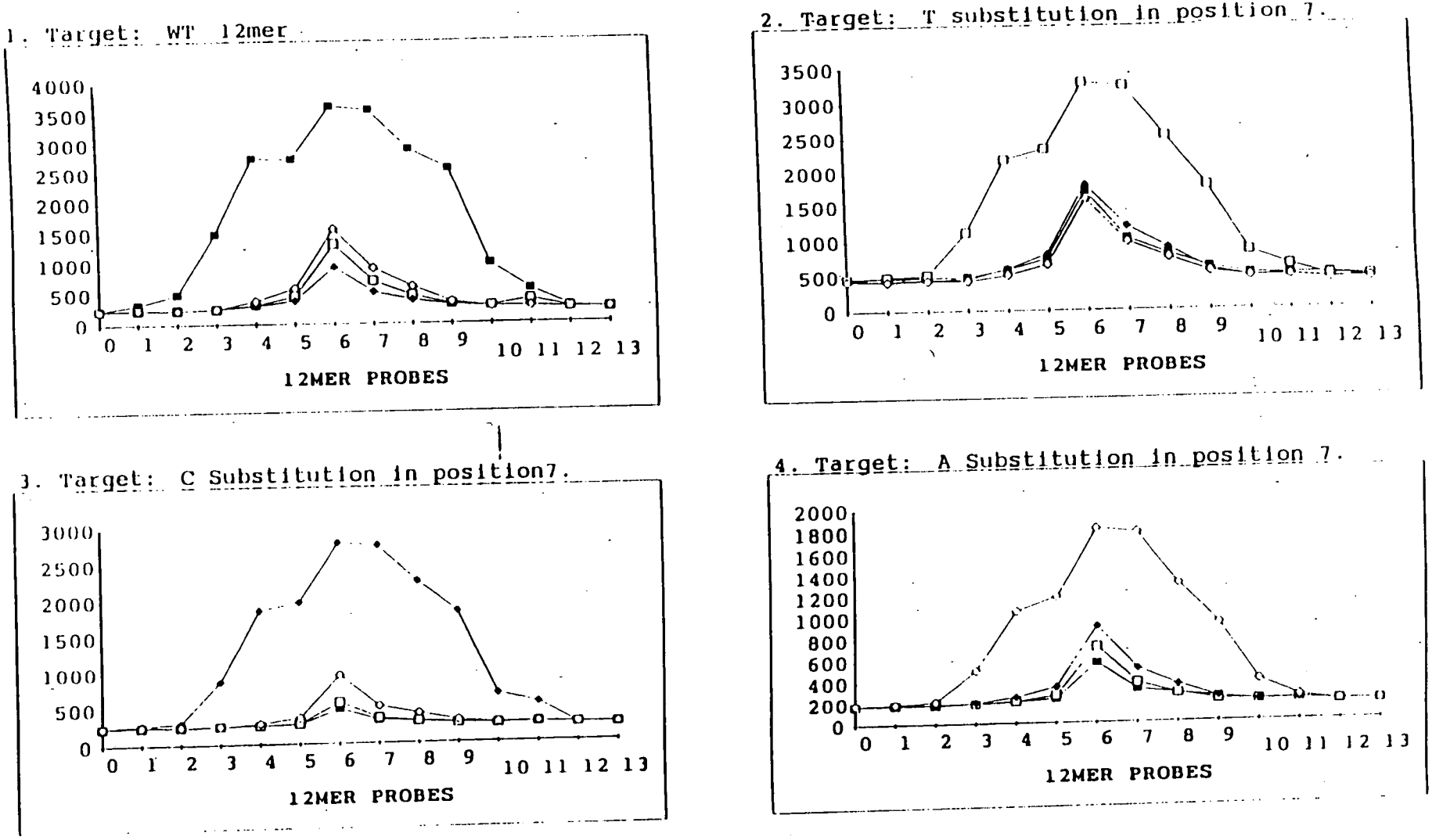
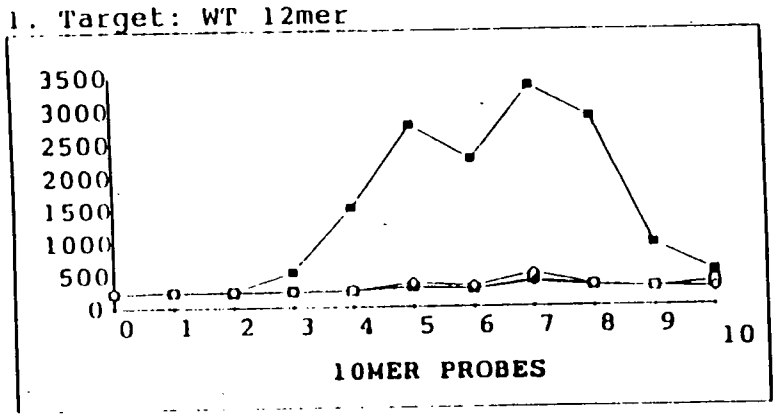
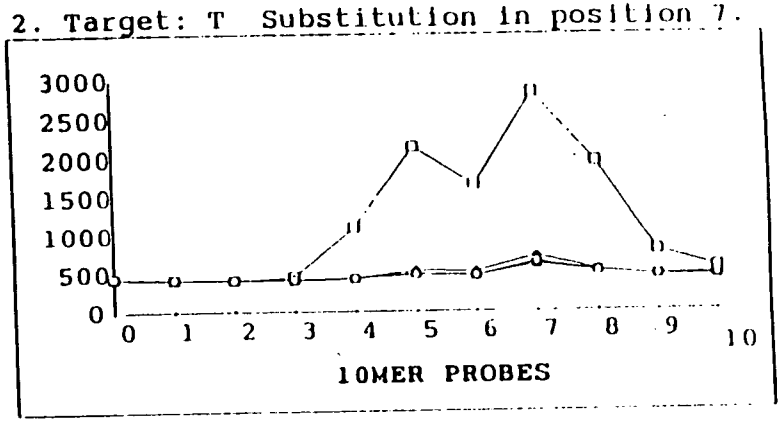
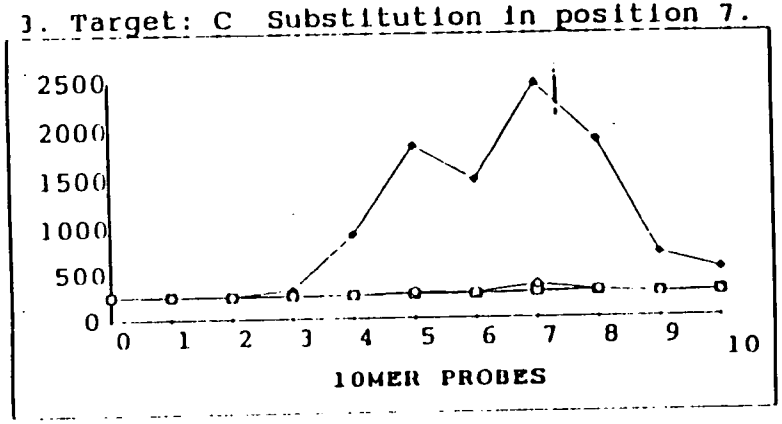


Fig. 30

P53 EXON 6 CODON 192 REGION







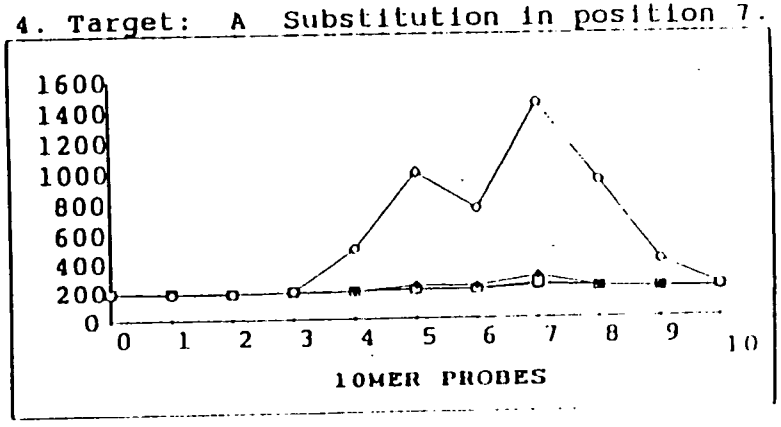


Fig. 32

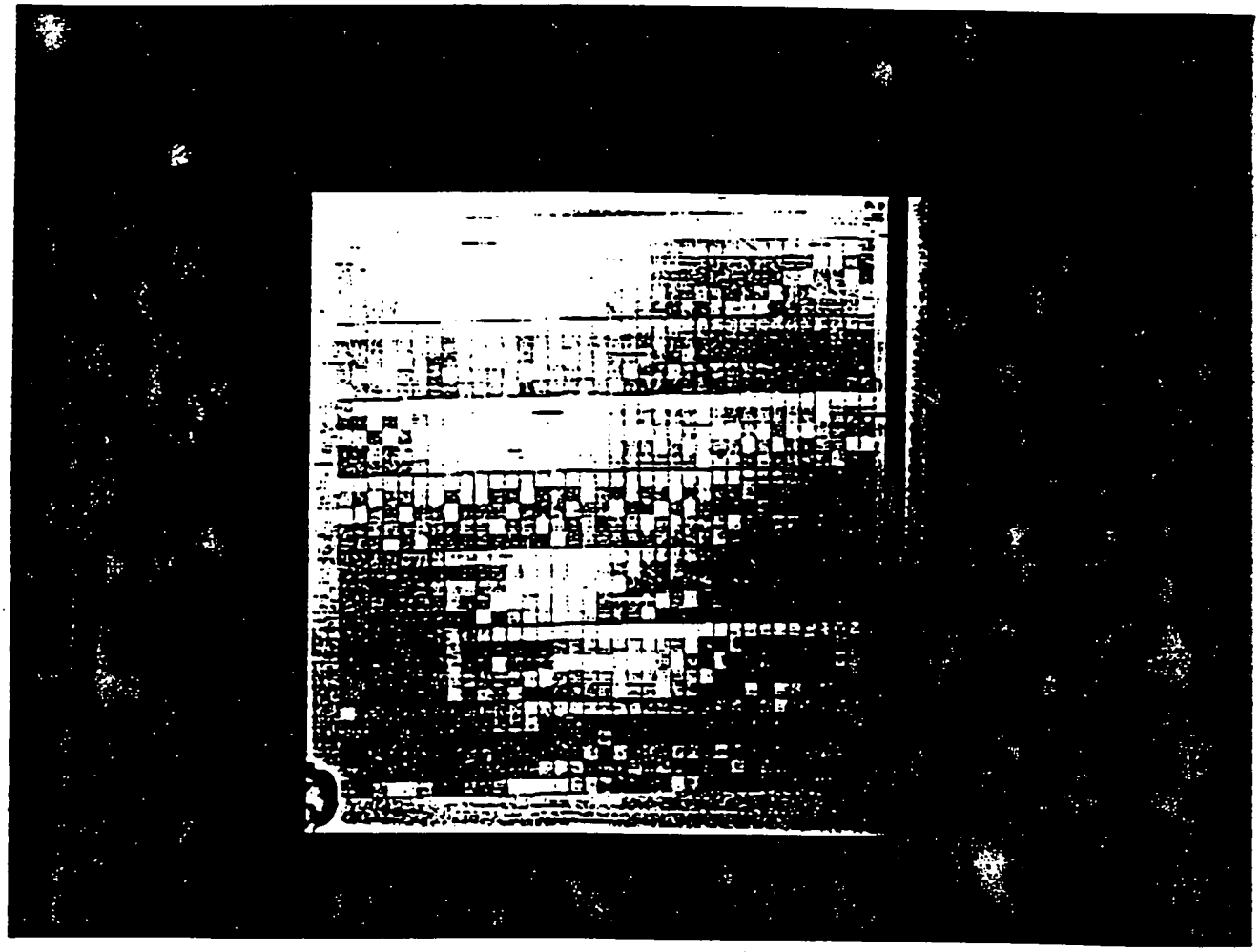


Fig. 33

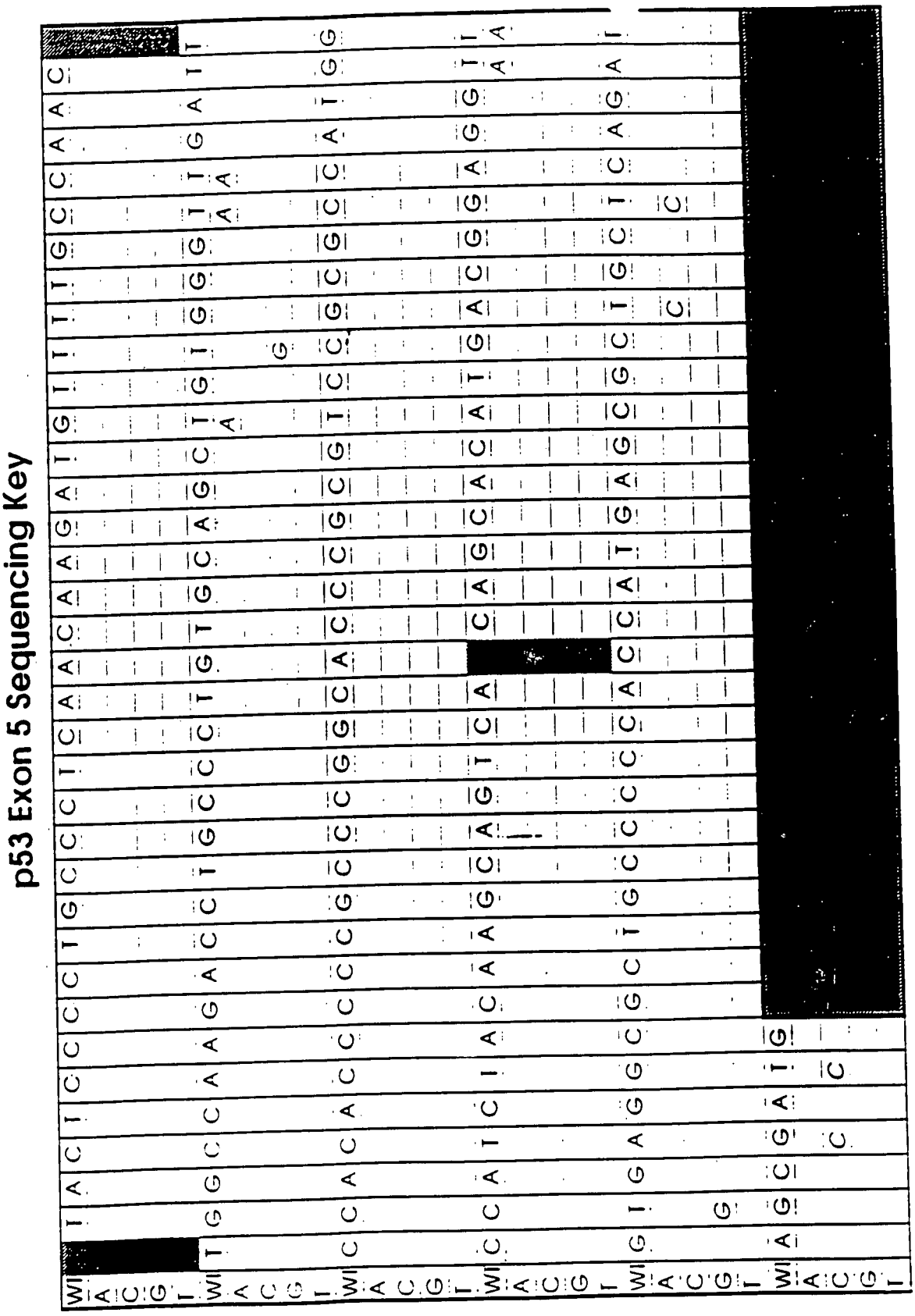


Fig. 34

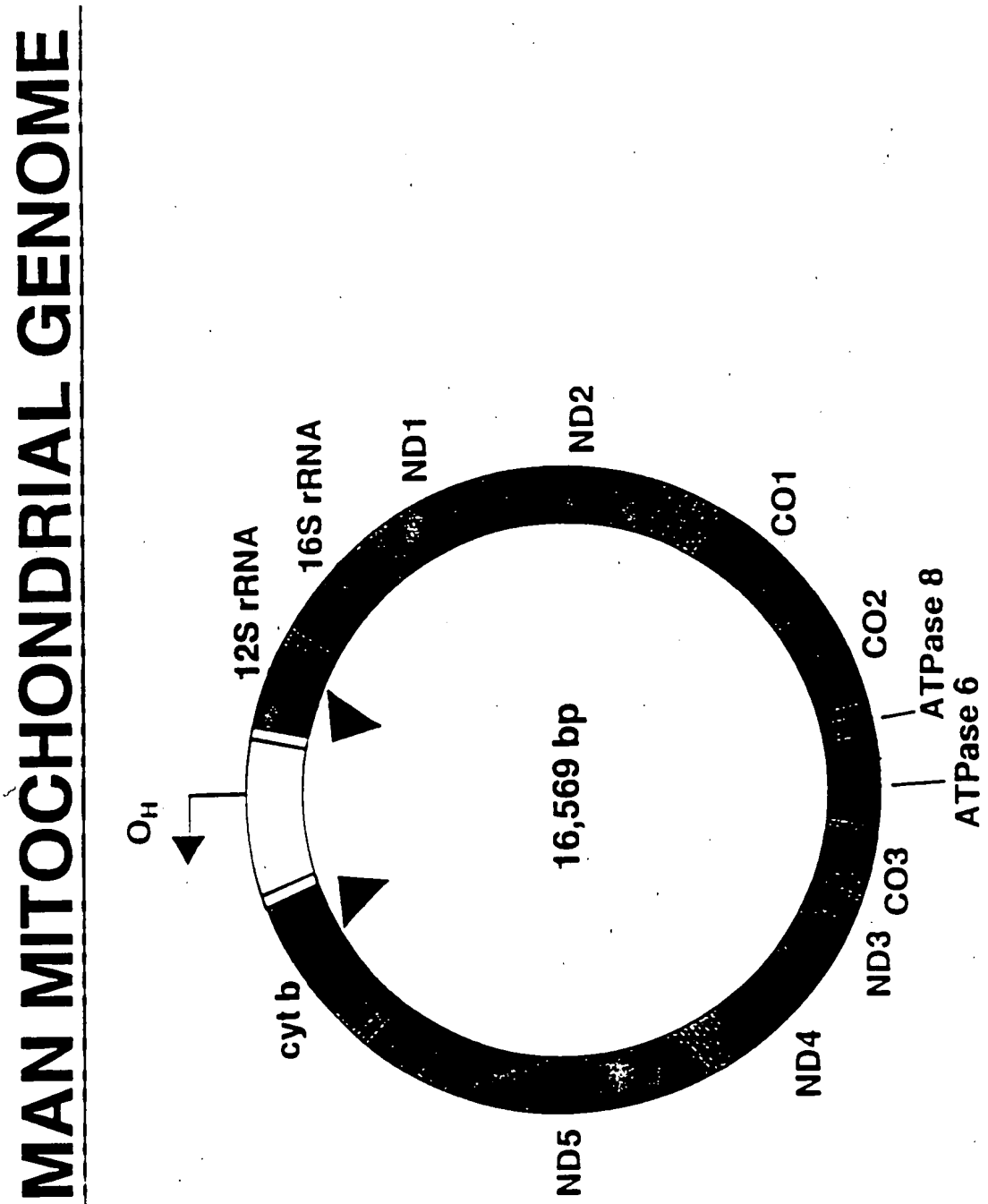

THE HUMAN MITOCHONDRIAL GENOME
12S rRNA
16S rRNA
ND1
ND2
CO1
CO2
ATPase 8
ATPase 6
CO3
ND3
ND4
ND5
cyt b
O_H
16,569 bp

Fig. 35

HYBRIDIZATION

mt4

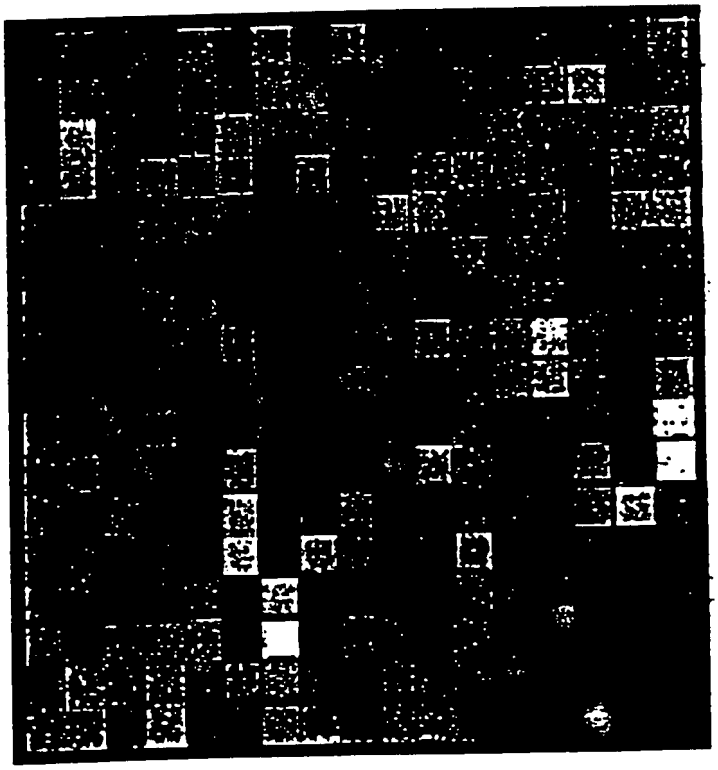


Fig. 36

HYBRIDIZATION

m15

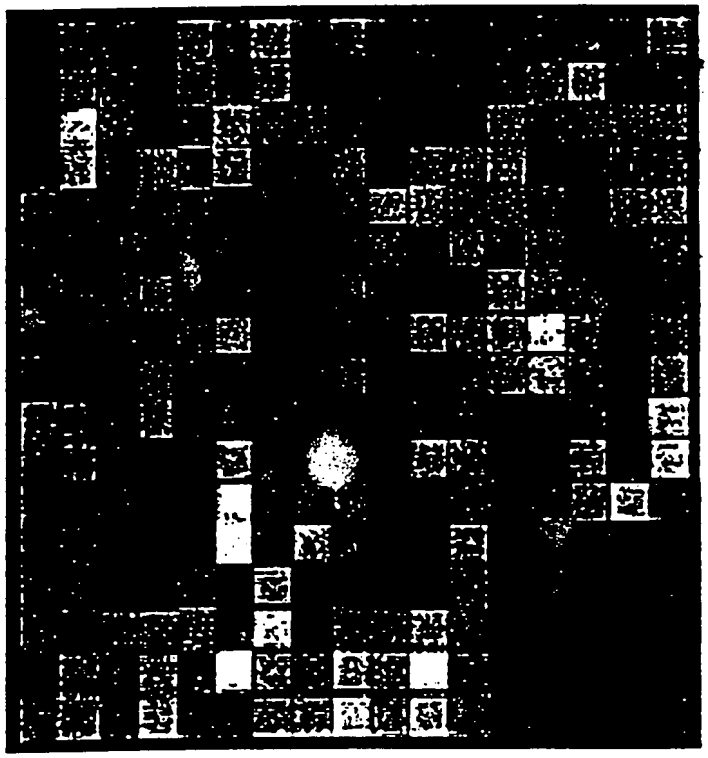


Fig. 37

PREDICTED DIFFERENCE IMAGE

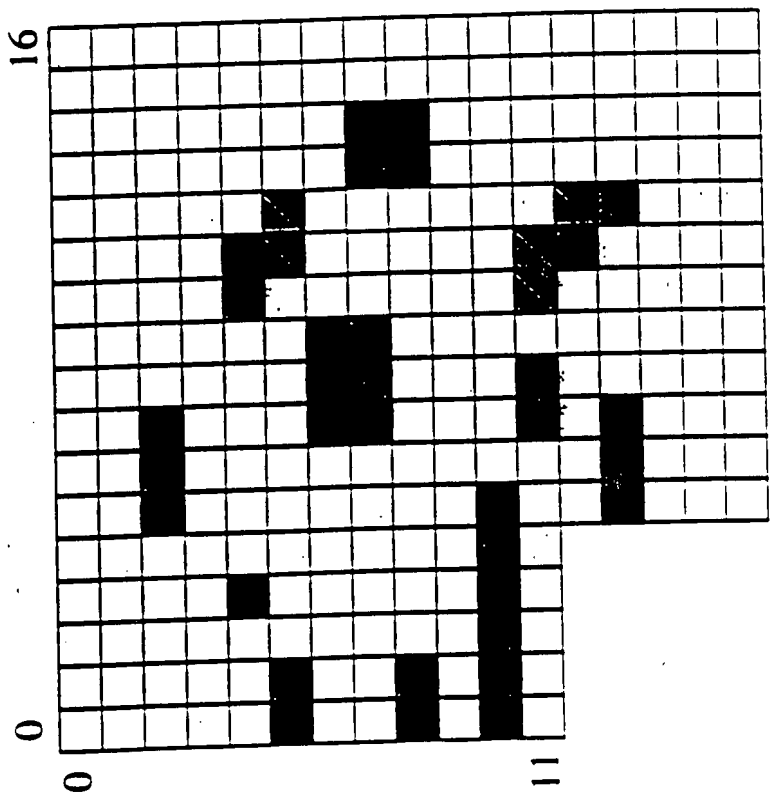


Fig. 38

DIFFERENCE IMAGE

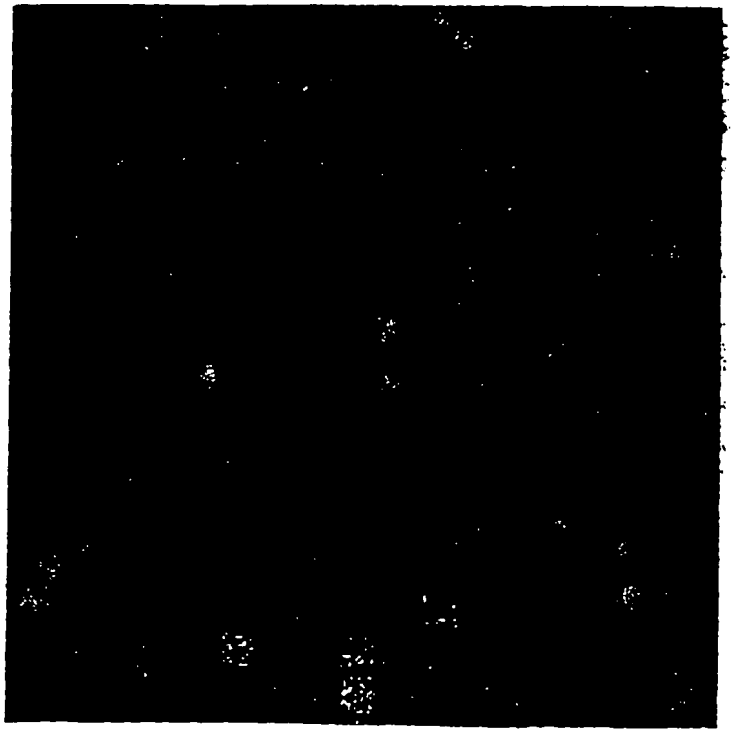
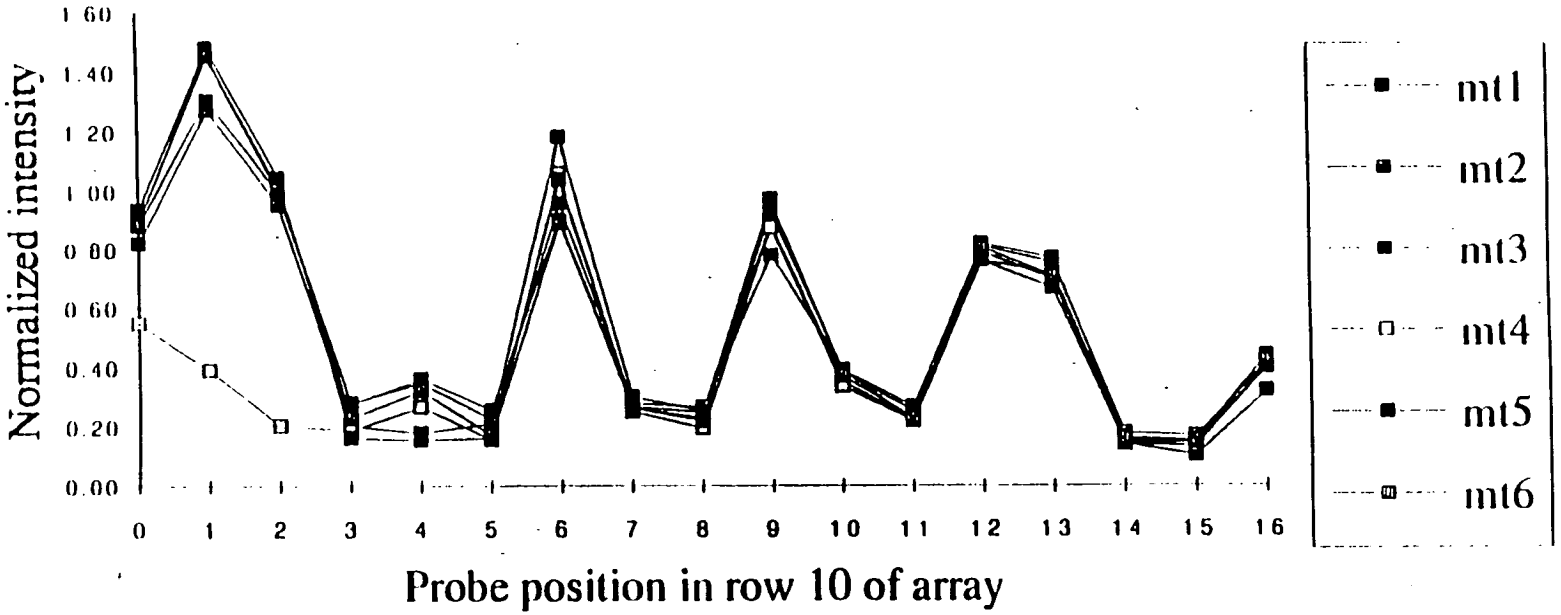


Fig. 39

NORMALIZED INTENSITIES

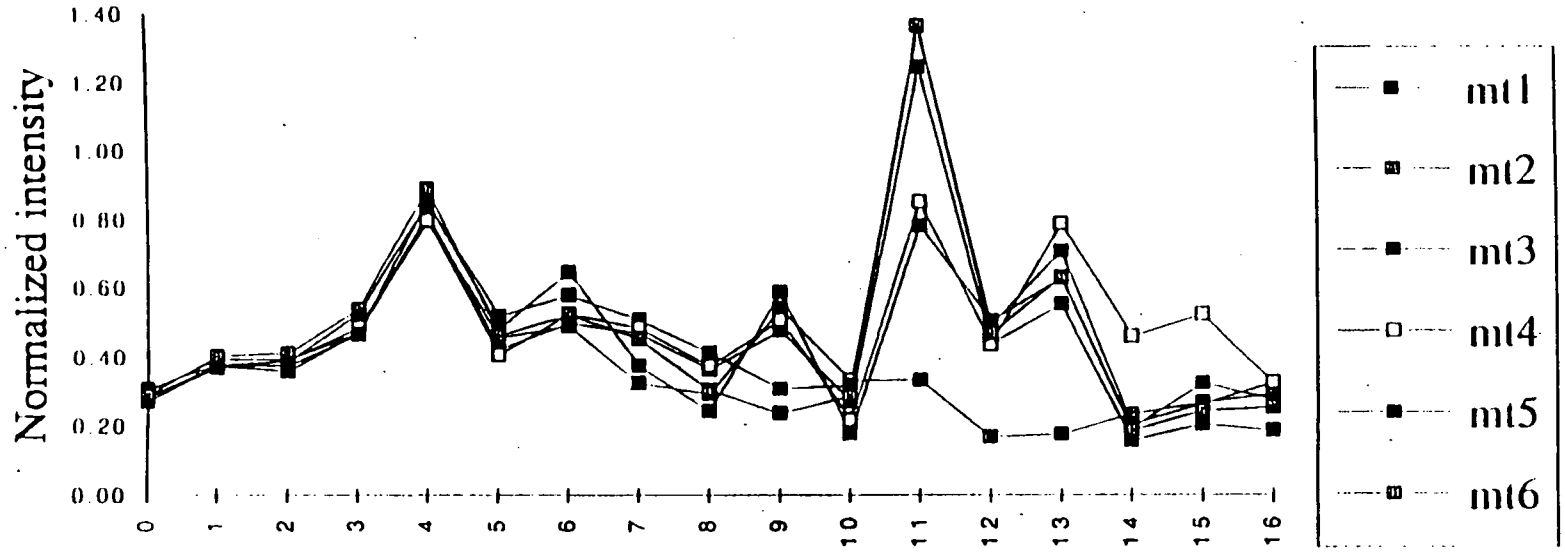


probe position	0	1	2	3	4	5
probe length	13	13	12	12	12	12
sample (mt1 -> 6)	4	4	4	2, 5	2, 5	2, 5
mismatch position from 3' of probe	12	5	3	12	7	2
base change	t -> a	t -> a	t -> a	t -> c	t -> c	t -> c

Fig. 40
Sheet 1 of 2

WO 95/11995
PCT/US94/12305

NORMALIZED INTENSITIES



probe position	6	7	8	9	10	11	12	13
probe length	13	12	12	13	14	13	12	12
sample (mt1 -> 6)	2	2, 5	2, 5, 6	3, 6	3, 4, 5	2, 4, 5	2	2
mismatch position from 3' of probe	13	9, 10	3, 4 11	11, 5	4, 11, double	11, 3, double	6	3
base change	c -> t	c -> t	c -> t t -> c	t-> c	t -> c double	g -> a t -> c double	g -> a	g -> a

Fig. 40
Sheet 2 of 2

WO 95/11995
PCT/US94/12305

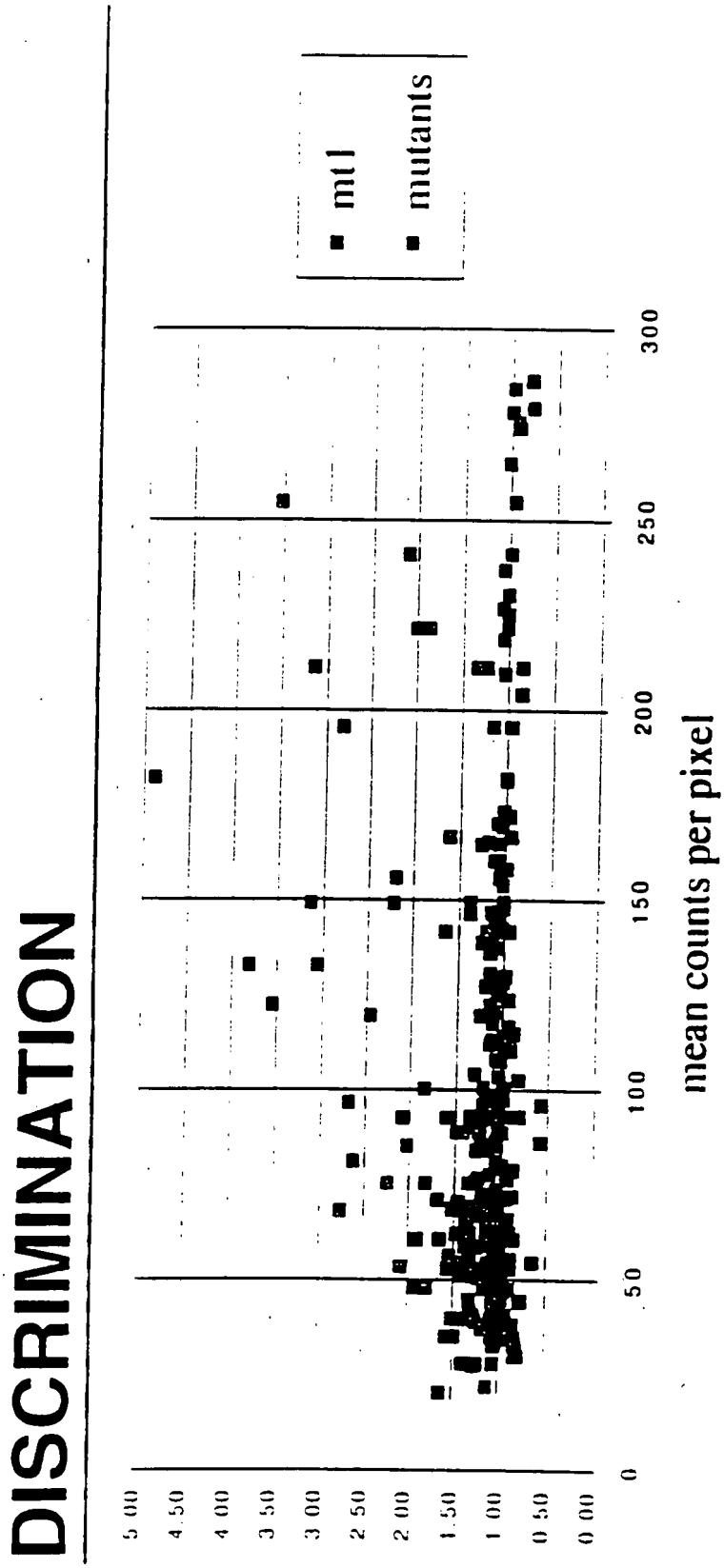

DISCRIMINATION
mtl
mutants
mean counts per pixel
0
50
100
150
200
250
300
0 00
0 50
1 00
1 50
2 00
2 50
3 00
3 50
4 00
4 50
5 00

Fig. 41

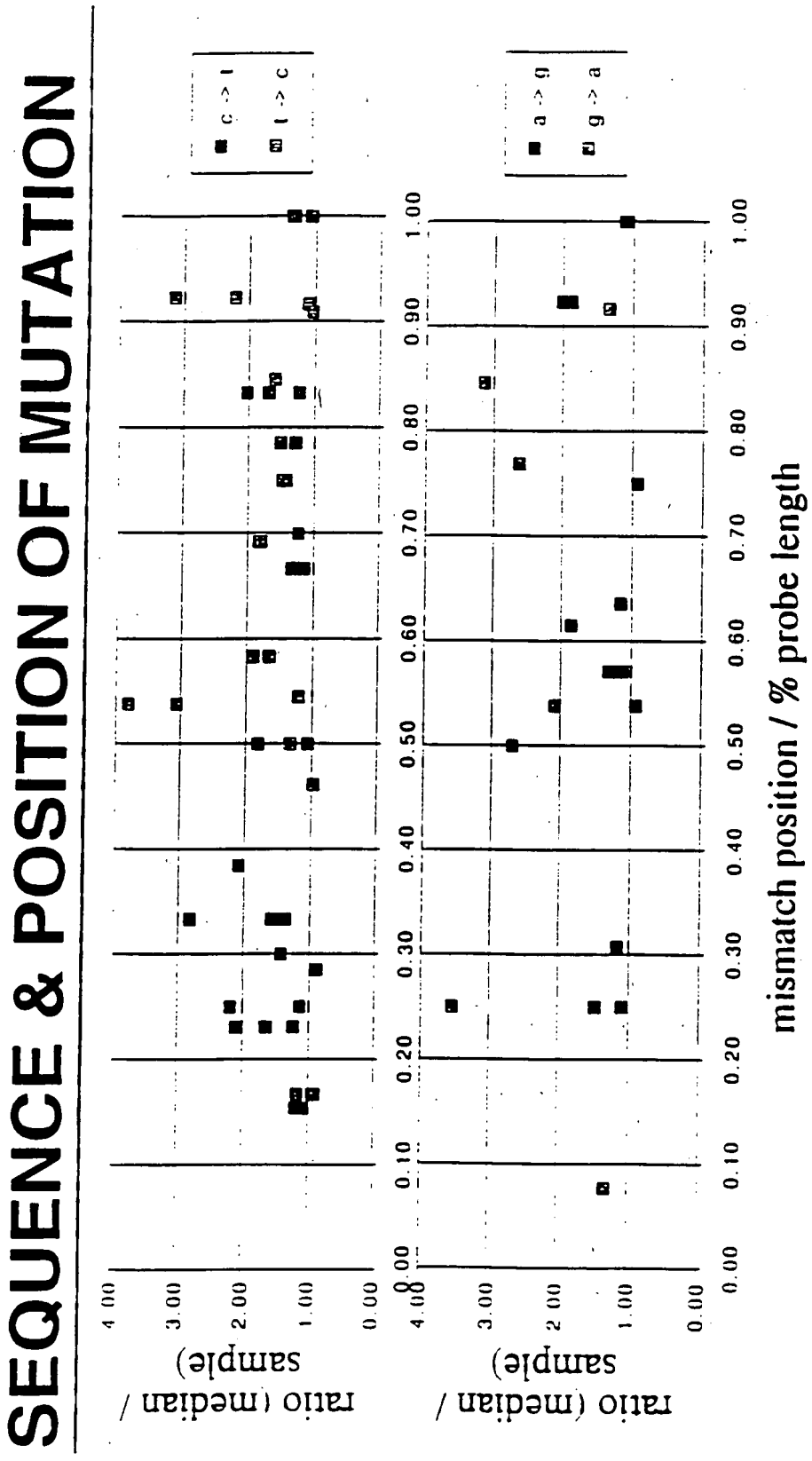

SEQUENCE & POSITION OF MUTATION
c -> t
t -> c
a -> g
g -> a
ratio (median / sample)
ratio (median / sample)
mismatch position / % probe length
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
0.00
1.00
2.00
3.00
4.00

Fig. 42

SEQUENCE

1 50

XaacaaacctacccaccttaacagtacataaagccatttacX

cgtacatagcacattacagtcaaatccccttcgtccccatggatgaccc

ccctcagatagggtcccttgaccaccatcctcccgtgaaatcaatatccc

gcccaagagtgctactcctcgctcccgggcccataacacttgggggtagg

ctaaagtgaactgtatccgacatctgggttcctcacttcaggg**T**cataaagcc

ctaaatagcccacacgttcccctaaataagacatcacgatggatccacag

gtctatcacccttattaaccactcacgggagctctccatgcattttggtatt

ttcgtcctgggggtatgcacgcgatagcattgcgagacgctggagccggga

gcaccctatgtcgcagtatctgtcttgattcctgcctcat**T**cc**T**attatt

tatgcgcacctacgttcaatattacaggcgaacatacttactaaagtgtgt

ttaattaattaatgcttgtaggacataataattaacaattgaatgtctgcac

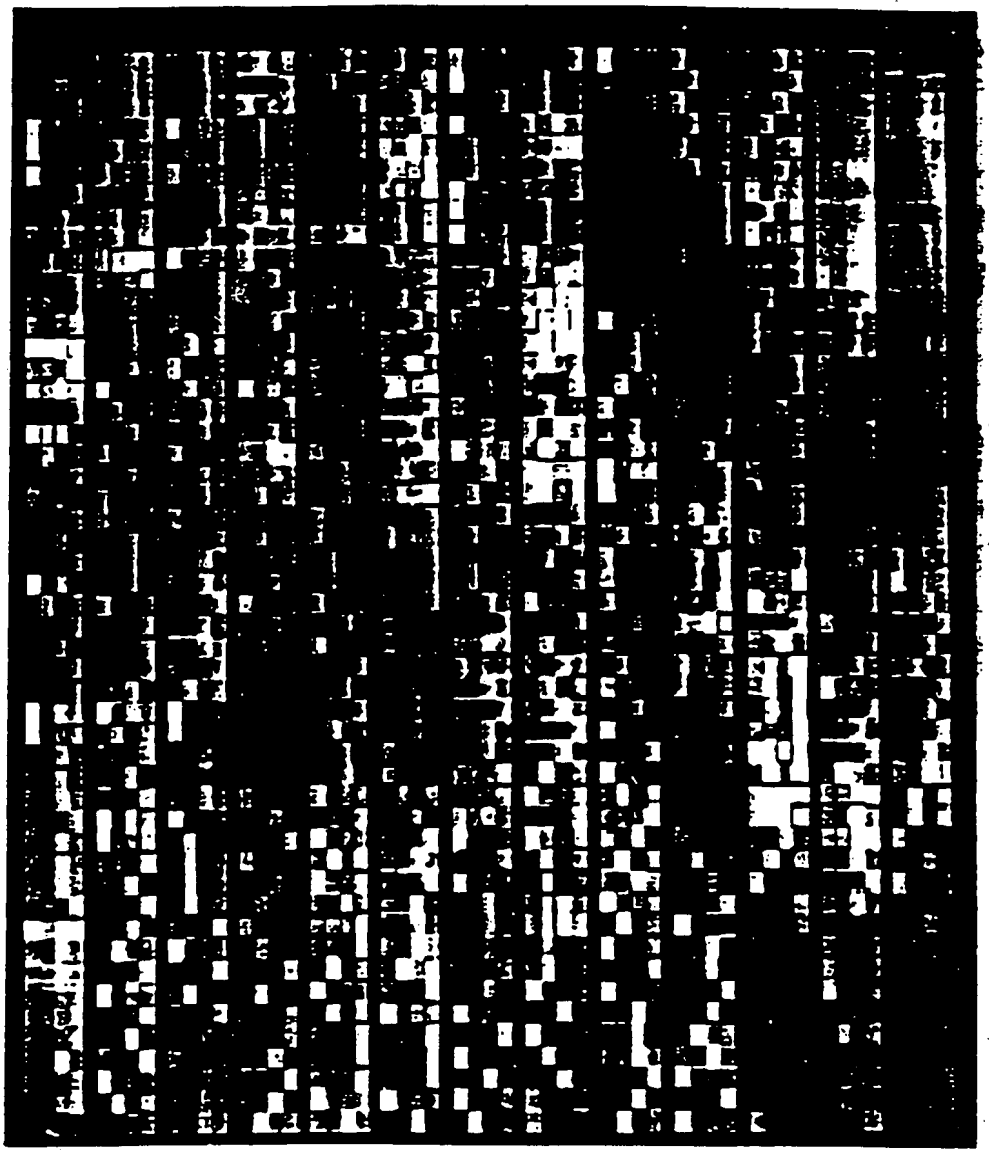
agcc**A**cttttccacacagacatcataacaaaaaattttccaccaaaccccc

X**c**tcccccgcttcctggccacagcacttaaacacatc**T**ctgccaaacccc**X**

Fig. 43

50 57

Fig. 44



HYBRIDIZATION

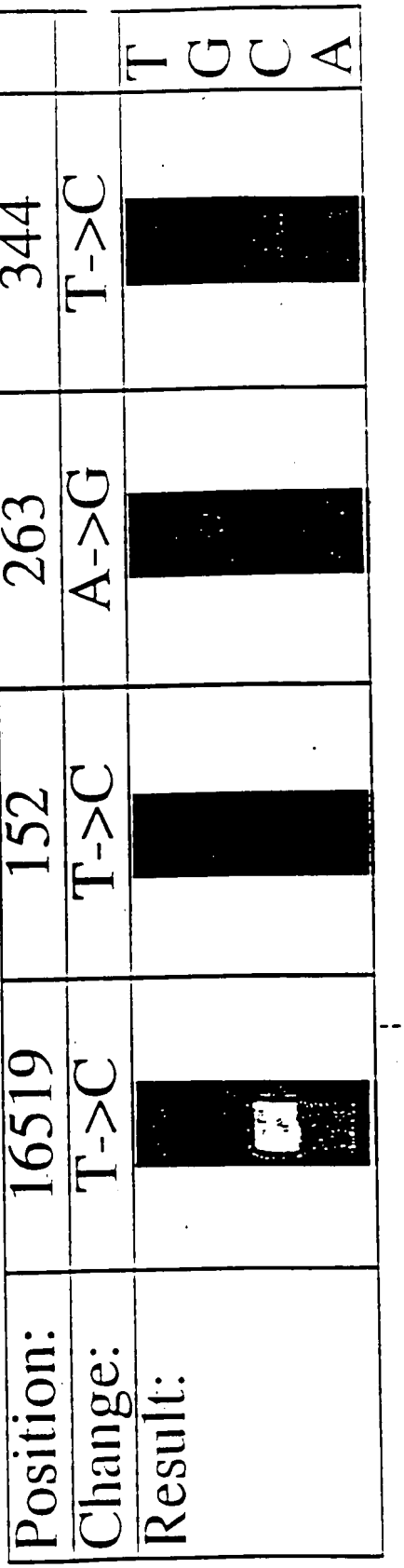


Fig. 45

Fig. 46

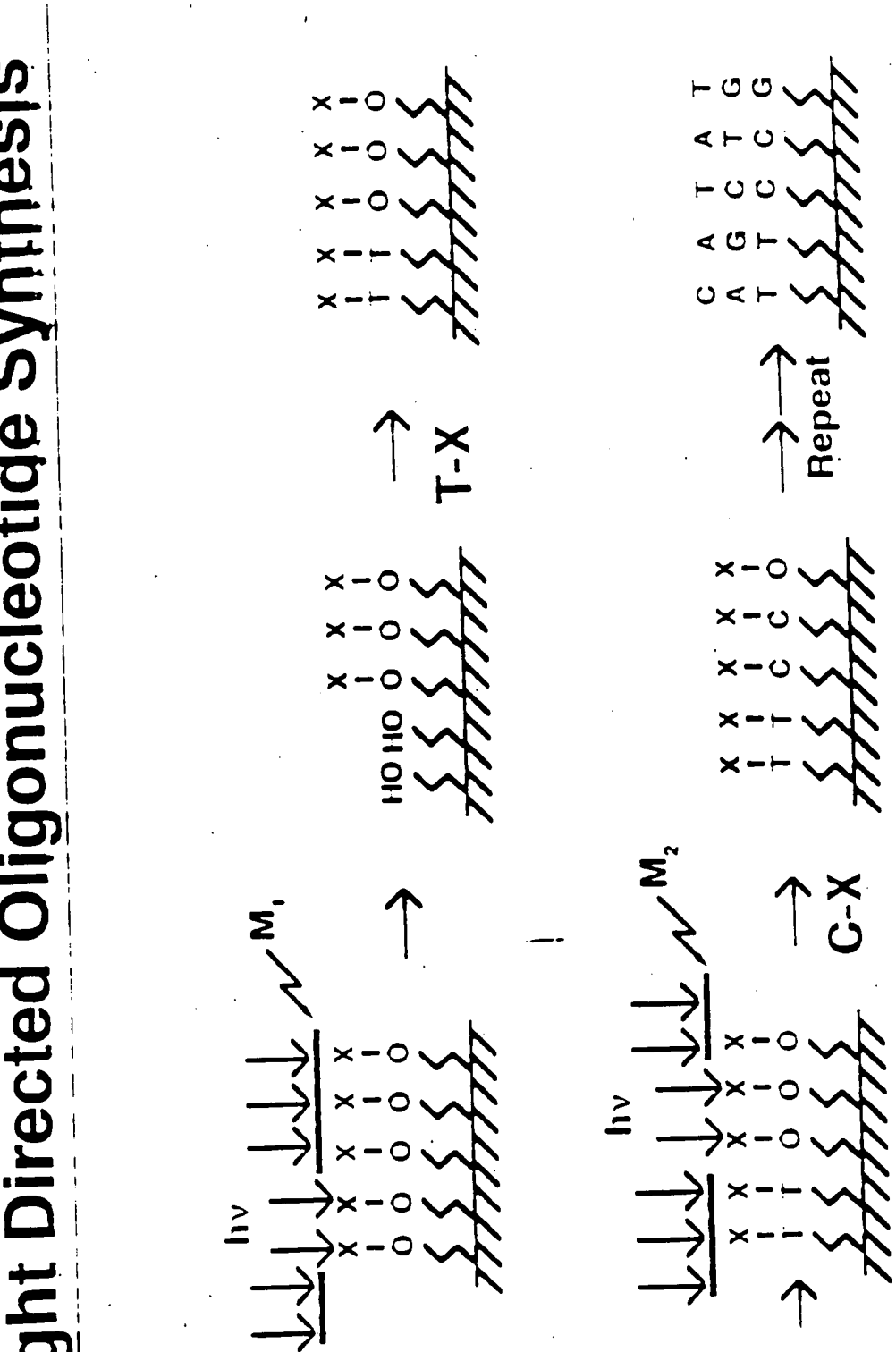

Light Directed Oligonucleotide Synthesis
hν
M1
T-X
C-X
M2
hν
Repeat

Fig. 47

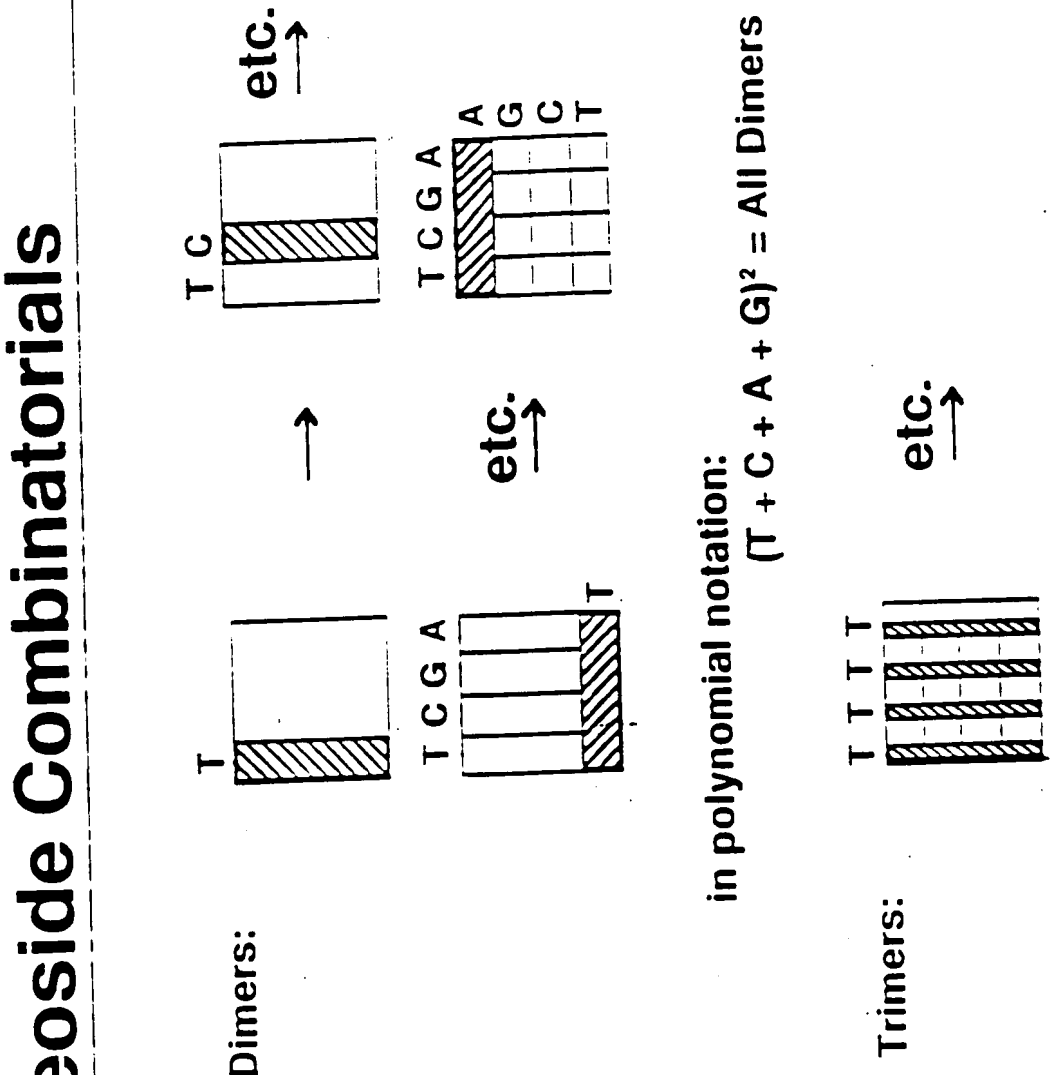

Nucleoside Combinatorials
Dimers:
T
T C
etc.
T C G A
T C G A
A G C T
etc.
in polynomial notation:
(T + C + A + G)² = All Dimers
Trimers:
T T T T
etc.

Fig. 48

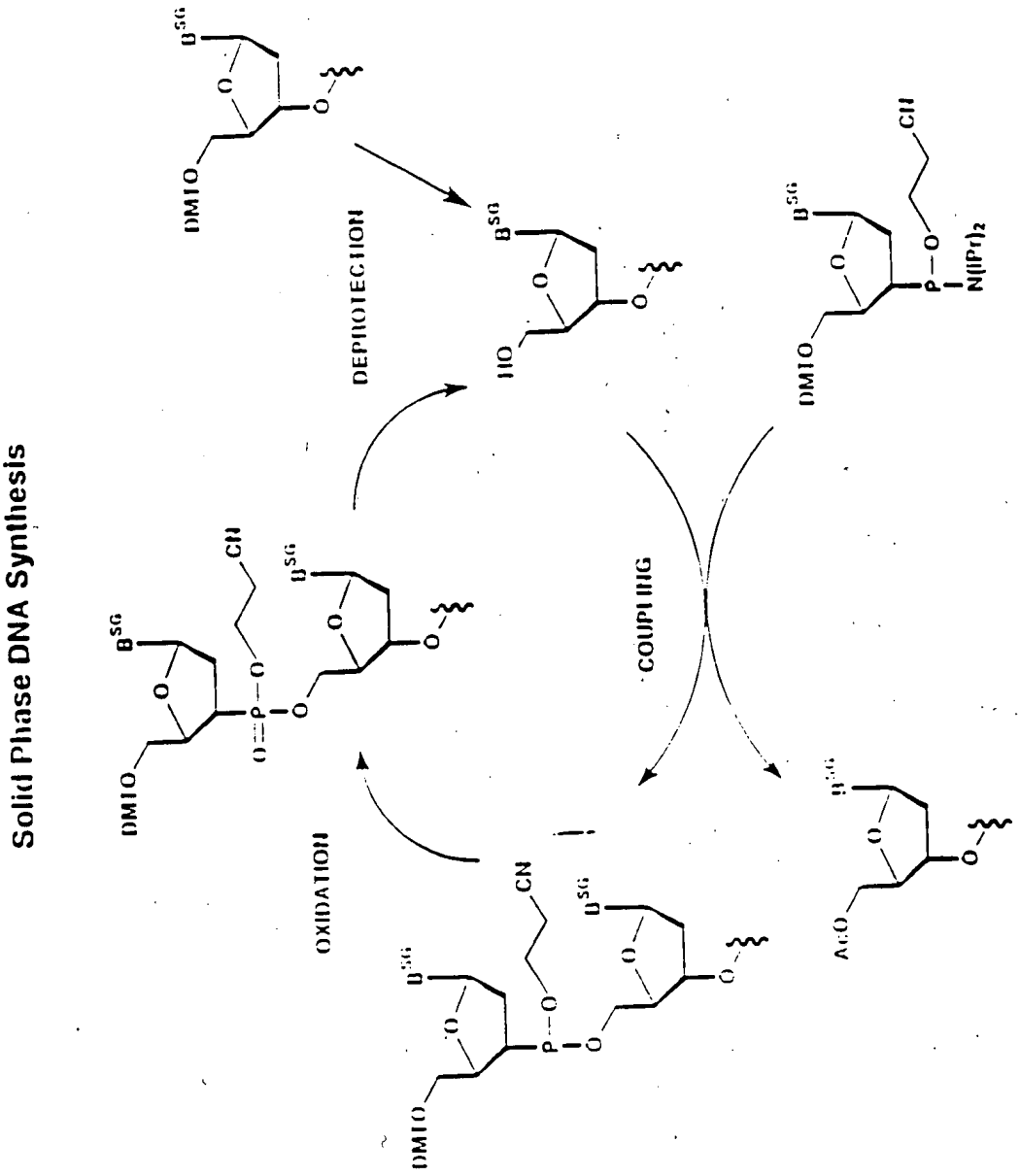

Solid Phase DNA Synthesis
DEPROTECTION
COUPLING
OXIDATION
DMTO
HO
CN
N(iPr)2
B^SG

Fig. 49

Nucleoside Buildingblocks
MeNPOC-Cl
Pyridin, 4°C
DIEA, DCM
dC-iBu
dT
dG-Pac
dA-Pac

Fig. 50

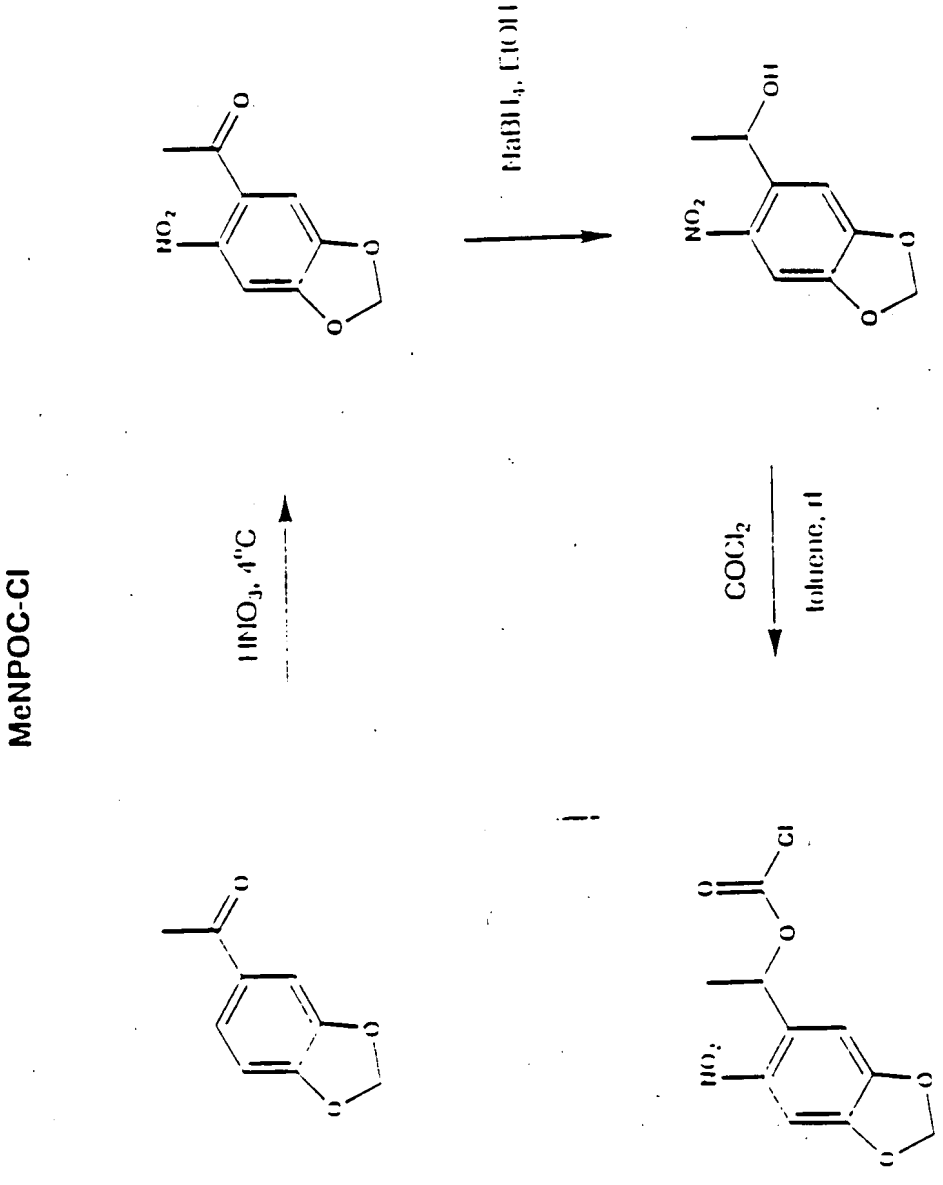
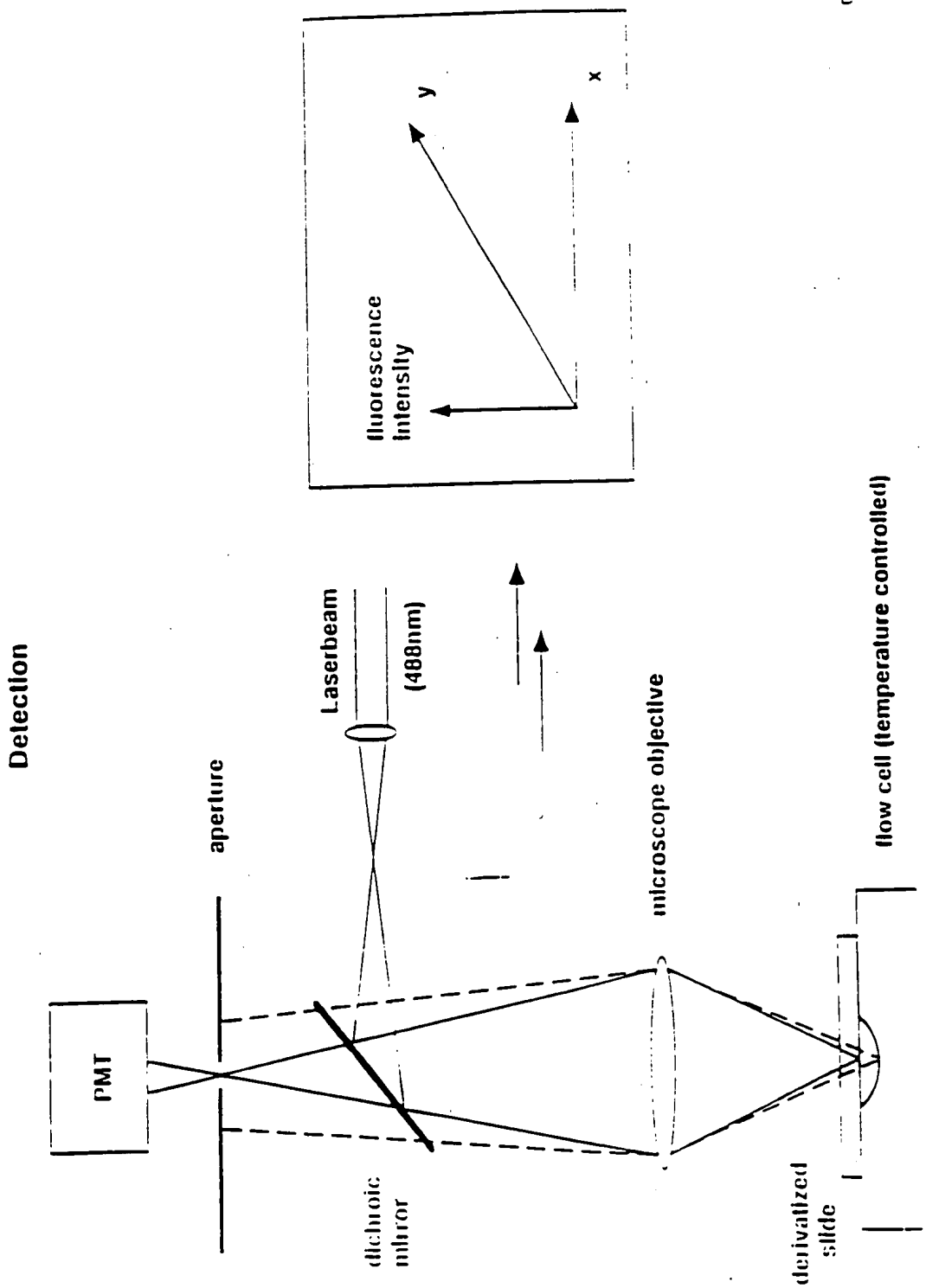


Fig. 51


fluorescence intensity
y
x
Detection
PMT
aperture
dichroic mirror
Laserbeam
(488nm)
microscope objective
flow cell (temperature controlled)
derivatized slide